

**EFFECT OF ACCELERATED DRYING ON THE REDUCTION OF *Salmonella*
ON ALMONDS BY THERMAL AND ELECTRON BEAM IRRADIATION
PASTEURIZATION TREATMENTS**

A Thesis

by

ZAHRA HASSAN MOHAMMAD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Alejandro Castillo
Committee Members,	Elsa Murano
	Rosana Moreira

Head of Department,	Jimmy Keeton
---------------------	--------------

August 2013

Major Subject: Food Science and Technology

Copyright 2013 Zahra Hassan Mohammad

ABSTRACT

After two outbreaks of salmonellosis that were linked to the consumption of almonds in 2001 and 2004, scientific community and industry have encouraged the study of pathogen inactivation kinetics in almonds. However, these studies often have overlooked the increase of water resulting from the inoculation of almonds. This increase of free water may result in data that may be overestimating the final outcome of treatments applied to pasteurize almonds in industrial settings. Therefore, in 2011 a study of almonds was performed at Texas A&M University to determine whether there was a need for Aw restoration. In this study, the effect of accelerated drying on the reduction of *Salmonella* on almonds was investigated, *Salmonella* Enteritidis PT 30 isolated from one of the outbreaks linked to almonds, and *Salmonella* Typhimurium LT2 were used. For irradiation, the effect of vacuum packaging and air packaging on the sensitivity of *Salmonella* to e- beam irradiation was evaluated for both accelerated drying and conventional drying. The D value for *Salmonella* Enteritidis PT 30 when treated with blanching at 88°C with accelerated drying was 10.7 ± 0.1 s, and 12.8 ± 0.3 s with conventional drying. When subjected to oil roasting at 127°C, the D values were 10.5 ± 0.1 s with accelerated drying, and 10.2 ± 0.2 s with conventional drying. For irradiation treatments, the D₁₀ value for vacuum packaged almonds was 0.35 ± 0.02 kGy with accelerated drying, and 0.38 ± 0.04 kGy with conventional drying. When irradiated in the presence of air, the D₁₀ value of *Salmonella* was 0.26 ± 0.04 kGy for almonds with accelerated drying, and 0.29 ± 0.03 kGy conventional drying. In conclusion, the

accelerated drying process resulted in greater reduction in *Salmonella* on almonds in comparison to conventional drying when subjected to blanching but no differences were found for oil roasting ($P > 0.05$). For e-beam irradiation the D_{10} values were significantly greater ($P < 0.05$) for vacuum with accelerated drying than for Air almonds with accelerated drying. This indicates that if a process applied in the industry were to be developed based on challenge studies when conventional drying was achieved, the almonds might likely be subjected to a treatment that is stronger than necessary to achieve pasteurization.

.

DEDICATION

To my husband for his unconditional support

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Alejandro Castillo, and my committee members, Dr. Elas Murano and Dr. Rosana Moreira, for their guidance and support throughout the course of this research.

I also express my gratitude to:

All my friends and labmates, for their technical assistance and moral support throughout this study, especially to Mariana Villarreal, Keila Perez, Songsirin Rnengrishes, Veronica Arias, Katie Kirsch, Wendy Alvarez and Elizabeth Duarte

Thanks also go to the department faculty and staff for making my time at Texas A&M University a great experience. I also want to extend my gratitude to Borlaug Institute for the economic support during this study.

Special thanks to Ministry of Agriculture and Ministry of Higher Education in Kurdistan Region for their support to take this opportunity.

Finally, I want to thank my family, for their constant encouragement and to my husband for his patience and support.

TABLE OF CONTENTS

	Page
ABSTRACT.....	ii
DEDICATION.....	iv
ACKNOWLEDGMENTS.....	v
TABLE OF CONTENTS.....	vi
LIST OF FIGURES.....	viii
LIST OF TABLES.....	ix
CHAPTER I INTRODUCTION AND LITERATURE REVIEW.....	1
Introduction.....	1
Literature review.....	4
Almond history.....	4
Almond board of California.....	4
Almond health benefits.....	5
Consumption statistics	5
The optimum storage condition of almonds.....	6
Sources of <i>Salmonella</i> in almonds.....	7
Characteristics of <i>Salmonella</i> bacteria.....	9
Salmonellosis.....	9
Low Aw products and <i>Salmonella</i> outbreaks.....	10
Almonds.....	11
Chocolate and coconut.....	12
Peanut.....	13
Pine nut.....	15
Sesame seed or helva.....	16
Tahini and helva.....	16
Control of <i>Salmonella</i> in low moisture food.....	16
Good agriculture practices.....	18
Mechanisms for <i>Salmonella</i> survival and resistance.....	20
Thermal pasteurization of almonds.....	21
Blanching.....	22
Oil roasting.....	23
Other thermal treatments.....	24

Non-thermal pasteurization.....	26
Electron beam irradiation.....	29
CHAPTER II MATERIALS AND METHODS.....	32
Materials and methods.....	32
Almond acquisition and storage.....	32
Bacterial culture.....	32
Preliminary study.....	33
Accelerated drying.....	35
Inoculum preparation.....	36
Inoculation of almonds.....	37
Sample preparation.....	38
Blanching.....	39
Oil roasting.....	40
Electron beam irradiation.....	40
Microbiological analysis.....	43
Statistical analysis.....	43
CHAPTER III RESULTS AND DISCUSSION.....	44
Results and discussion.....	44
Preliminary study.....	44
Water activity, moisture and accelerated drying.....	45
Thermal reduction of <i>Salmonella</i>	53
Blanching.....	53
Oil roasting.....	54
Decimal reduction dose of <i>Salmonella</i> on inoculated almonds using electron beam irradiation.....	59
Irradiation.....	59
CHAPTER IV CONCLUSIONS.....	68
REFERENCES.....	70

LIST OF FIGURES

	Page
Figure 1: The survival of <i>Salmonella</i> after drying procedures (with accelerated and conventional drying.....	50
Figure 2: Thermal inactivation curves for blanching with accelerated and conventional drying	56
Figure 3: Thermal inactivation curves for roasting with accelerated and conventional drying.....	57
Figure 4: The effect of accelerated drying on reduction of <i>Salmonella</i> to treatments.....	58
Figure 5: Comparison between vacuum with accelerated and conventional drying	64
Figure 6: Comparison between air with accelerated and conventional drying	65
Figure 7: The effect of vacuum and air atmosphere on the sensitivity of <i>Salmonella</i> to electron beam Irradiation.....	66
Figure 8: The effect of accelerated drying on reduction of <i>Salmonella</i> to electron beam irradiation.....	67

LIST OF TABLES

	Page
Table 1: The average of cooling temperature for both blanching and oil roasting	42
Table 2: <i>Salmonella</i> reduction over the time in different media with accelerated drying.....	47
Table 3: <i>Salmonella</i> reduction over the time in different media with conventional drying	48
Table 4: Water activity measurement with accelerated and conventional drying	49
Table 5: The survival of <i>Salmonella</i> after accelerated and conventional drying(n=4)	51
Table 6: The moisture content for almonds with accelerated and conventional drying.....	52
Table 7: The calculated dose for spot inoculated samples.....	63

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

In recent years, the global demand for almonds has increased dramatically due to better consumers' awareness of almond health benefits. In the USA almonds are one of the products with the greatest- production and are considered one of the most important economical sources, in 2008 growers in California produced 1.590 billion pounds of almonds that worth about billions of dollars (ABC 2009). Like most nuts, almonds are of low moisture; therefore do not support the growth of microorganisms. However, *Salmonella* can recover and survive at extreme environmental conditions such as low water activity (Podolak 2010, Betts and R. 2007). Two outbreaks of salmonellosis have been linked to the consumption of raw almonds in the USA. The first outbreak occurred in 2001 and 2002 and the second outbreak, which was extended USA and Canada occurred in 2003-2004(MMWR 2004, Chan et al. 2002; Isaacs et al. 2005, CDC 2004). For these two outbreaks, the *Salmonella* associated with the outbreaks was *Salmonella* serovar Enteritidis phage type (PT) 30 (Danyluk et al. 2005; Uesugi et al. 2006). The mechanism for the presence of *Salmonella* in almonds is still unknown. However, many studies demonstrated that almonds may be contaminated during harvesting and post harvesting from the environment especially from the soil (Danyluk et al. 2008). In response to almonds outbreaks Federal Law mandates all almond processors in California should pasteurize their almonds in order to achieve at least 4-log reduction of

Salmonella bacteria before reaching to consumers in North America (U.S., Canada, and Mexico). The purpose of this pasteurization program is to ensure that consumers are provided with safe, good food products, free from pathogenic bacteria that can cause illness, without affecting the almond qualities (ABC 2012a). Therefore, all pasteurization technologies are evaluated by the Almond Board of California (ABC) Technical Expert Review Panel (TERP) for their ability to provide the required minimum of 4-log reduction in *Salmonella* contamination and to demonstrate no significant effect on the sensory, quality and nutritional characteristics of almonds (ABC 2007), the regulation took effect on September 1, 2007 (Federal Register 2007). The aim of this pasteurization process is to inactivate pathogenic and spoilage microorganisms in order to produce a safe and shelf stable product of the raw almonds (Da Silva and Gibbs 2009; Villa-Rojas et al. 2012).

However, in some of the relevant studies on almond pasteurization using both thermal and non- thermal interventions, the researchers seem to have overlooked the increase of water resulting from inoculation of almonds, and this increase in free water may affect the final outputs of data that might cause overestimates of time need for inactivation of *Salmonella* (Chang et al. 2010, Bari et al. 2009, Bari et al. 2010, Pao et al. 2006, Jeong et al. 2009, Danyluk et al. 2005, Willford et al. 2008, Lee et al. 2006, Deng et al. 2007, Brandl et al. 2008, Uesugi et al. 2006, Harris L.J. et al. 2011, Goodridge et al. 2006, Yang et al. 2010). In 2011, Cuervo (2011) developed a method to restore the Aw to the baseline level after inoculation. This study indicated that even after the drying step described in the literature, the Aw of the almonds was still significantly

higher than the original Aw before inoculation. It was hypothesized that differences in the free water content on the surface of the almonds might affect the sensitivity of *Salmonella* to current thermal treatments for almond pasteurization and to electron beam (e-beam) irradiation (Cuervo 2011).

The objectives of the present work were to investigate the effect of accelerated drying on the sensitivity of *Salmonella* to thermal treatments such as hot water blanching and oil roasting, and to electron beam irradiation on inoculated almonds in comparison to inoculated almonds that were not subjected to accelerated drying, and to evaluate the effect of vacuum packaging vs. air packaging on the sensitivity of *Salmonella* to electron beam irradiation.

Literature review

Almond history

The almond tree is thought to have originated in China and central Asia. Almond is considered one of the oldest nut crops of south west Asia, and from Asia it has diffused to other regions and continents (G. Ladizinsky. 1999, Bruhn et al., 2010, Rosengarten 1984). Almonds were planted in Europe because of the nut's popularity. Since it thrived in warm climate of Spain and Italy and since California possesses a climate like the Mediterranean, almonds trees were brought to California in the mid-1700s by the Spanish Franciscans (Bruhn et al. 2010, Kester and Ross 1996, Boriss H. & Henrich B. 2012). Initially, almonds trees were planted in coastal areas, but they were not well suited to this cooler environment. In the 1800s, almonds trees were planted in the warmer valleys of California. Today California is the largest place in the North America where almonds are grown commercially. Today almonds are the most widely grown nut in California (Bruhn et al. 2010). Almonds are grown on 550,000 bearing acres, more than 6,000 almond growers and 115 almond processors in California (ABC 2005).

Almond board of California

The Almond Board of California was established in 1950 under the federal Marketing Order 981 (AMS 2010). It administers grower -legislated Federal marketing under the supervision of the USDA. The ABC mission is to establish a productive environment for the production and marketing of California almonds (ABC 2009).

Almond health benefits

All throughout the history, almonds have been known to have high benefits to health and provide a unique delicious taste. California almonds are cholesterol-free and low in saturated fat, and research is now showing that almonds may also help maintain a healthy heart. (ABC 2012b). After an extensive review of nutrition and health studies, the U.S. Food and Drug administration has affirmed that including nuts in a diet low in saturated fat and cholesterol may help reduce the risk of heart disease. Almonds and other nuts may contribute to health through their protein, dietary fiber, and unsaturated fat (Bruhn et al. 2010, ABC 2012d). Other study also concluded that almonds used as snacks in the diets of hyperlipidemic subjects significantly reduce coronary heart disease risk factors because of protein and fiber and the non saturated fat and presence of monounsaturated fatty acid components of the nut (Jenkins et al. 2002).). According to a recent report by ABC one ounce of California almonds contains about 35% daily value of vitamin E ups, 3.5 grams of dietary fiber, six grams of plant- based protein, 8% daily value of calcium, only one gram of saturated fat, and 13 grams of “good” mono and polyunsaturated fats (ABC 2012c).

Consumption statistics

Accordion to the last report of the Almond Board of California, California produces 81% of the world’s almonds and 100% of the U.S. commercial supply. In 2011/2012 California Almonds produced 2.02 billion pounds of almonds on 760,000 bearing acres. And in the same year, 104 handlers shipped a record-breaking 1.9 billion pounds of almonds, a 14% increase over the prior year. Overall, shipments increased to

most markets. The U.S. remains the single largest market for California Almonds, hitting a new record of 547 million pounds and accounting for 29% of shipments. The remaining 71% of shipments was destined for export markets. These markets, led by China, Spain, India and Germany accounted for 1.4 billion pounds, also a new record (ABC 2012d). Based on the ABC report, about 31% of the almonds are consumed in the U.S.; 69% is exported to global 90 countries. Domestic shipments were increased 35% from 291.7 million pounds in 2002/2003 to 394.8 million pounds in 2007/2008. California almond exports achieved a 25% increase from 690.6 million pounds in 2002/2003 to 866.4 million pounds in 2007/2008(ABC.2009).

The optimum storage condition of almonds

In general almonds have ability to tolerate the low temperature because of low water activity level and high fat of the kernel. Therefore, the optimum storage conditions should involve the condition that maintains the low water activity content. A water activity <0.7 at 25 °C is a safe water activity for almonds and other nuts based on federal regulations because it hinders microbial growth (John 2011). This author suggested that cold storage is useful for almonds and other nuts because it reduces lipid oxidation, almonds with shell can be stored for up to 20 months at 0 °C, 16 months at 10 °C, and 8 months at 20 °C, Also suggested that almonds should not be stored with products that have strong odors because almonds can absorb odors because of the high fat content. Kader (1996) suggested that storage RH to be 65% because too low a water contents may affect the flavor, color and texture.

Sources of Salmonella in almonds

Almonds can be potentially contaminated with *Salmonella* from environment during growing and harvesting season. During harvesting season, traditionally almonds are shaken from the tree and almonds left on the grounds for up to two weeks before collected and processed. Therefore, almonds can be easily become contaminated with pathogen while in ground from the environment especially the soil (Danyluk et al. 2008b, Reil et al. 1996). A study by Uesugi et al. (2007,) demonstrated the long-term environmental persistence of *Salmonella* Enteritidis PT 30 in one of the almond orchards associated with the outbreak. This was performed by drag swabs of almond orchard floors on three geographically linked farms in a 25-km² area. According to this study, *Salmonella* Enteritidis PT 30 was isolated over a period of 5 years from a single almond orchard. Danyluk et al. (2008b) also evaluated the factors potentially contributing to the long-term persistence of *Salmonella* enterica serovar Enteritidis phage type (PT) 30 in an almond orchard. This study demonstrated that *Salmonella* Enteritidis PT 30 is capable of extended survival (at least 180 days) in typical almond orchard soils even in the absence of growth substrates. Therefore, *Salmonella* Enteritidis PT 30 may survive in soils where almond hull nutrients are released. However, this study showed that temperature had a significant impact on the long-term survival of *Salmonella* Enteritidis PT 30 in soil, and also the present of nutrients from almond hulls has an impact on *Salmonella* growth. A study by Uesugi *et al.* (2006a) also showed the ability of *Salmonella* Enteritidis phage type (PT) 30 which associated with raw almonds outbreaks to growth in almond hull and shell. This study documented that *Salmonella* was able to survive in hulls dried at 15 and

37°C, and both hull and shell slurries supported rapid growth of *Salmonella* Enteritidis PT 30 at 24°C. Slurries containing hulls also supported growth at 15°C. This study also showed that risks of *Salmonella* contamination increased significantly with wetting almonds in the field through drop irrigation or rainfall, and the data provided evidence that the concentrations and risk of presence of *Salmonella* may increase with wet almonds. *Salmonella* Enteritidis PT 30 also could survive on almond kernels under a variety of common storage conditions for long periods (Uesugi et al. 2006b). Danyluk et al. (2008a) reported that *Salmonella* might migrate through the almond shell to the kernel under wet environments. This study indicated that the mechanism of migration of *Salmonella* from shell to kernel is wet environment that assist *Salmonella* to reach the kernel of almonds through water filtration, this study also indicated that even non motile strain of *Salmonella* can transfer from shell to kernel of almonds. However, this study has not shown whether the dry environment can prevent almonds from being contaminated, the study has just tested the wet environment. In this case, the mechanism of migration of *Salmonella* from shell to kernel should be tested with dry environment to determine whether this situation can prevent first: the contamination of almonds from *Salmonella*, second if contamination occurs, the dry environment should prevent the movement of *Salmonella* from shell to kernel. Therefore, a better knowledge is still needed about the advantage of keeping almonds in dry condition during post harvest and growing. In addition, studies focusing the conditions under dry environment are needed.

Another study by Danyluk et al. (2007) evaluated the prevalence and levels of *Salmonella* in the recalled product. These authors determined that *Salmonella* was

unevenly distributed within the product and that in most positive samples the levels of *Salmonella* were below the limit of detection. According to this study (Danyluk et al. 2007); the occasional presence of *Salmonella* is not considered a public health hazard in products that are subsequently heat treated prior to consumption. All these studies have documented that potential sources of almonds contamination with *Salmonella* are environment and soil.

Characteristics of Salmonella bacteria

Salmonella is a Gram-negative facultative anaerobic, oxidase negative, catalase positive rod-shaped bacterium in the family *Enterobacteriaceae*. *Salmonellae* live in the intestinal tracts of warm and cold blooded animals. *Salmonella* causes two diseases in humans salmonellosis and enteric fever (typhoid), due to bacterial invasion of the bloodstream, and foodborne infection/intoxication that causes acute gastroenteritis (Todar 2009). *Salmonella* is divided into two species, *Salmonella bongori* and *S. enteric*, and *Salmonella enteric* is divided into six subspecies, and only one of these *subspecies* responsible for illness in warm-blooded animals. *Salmonella* microorganisms were discovered by an American scientist named Salmon, and the *salmonella* called under his name (CDC 2013).

Salmonellosis

Salmonellosis is the most common foodborne infection caused by *Salmonella*. There are many different kinds of *Salmonella* bacteria. In the United States, *Salmonella* infection (salmonellosis) is caused by two *Salmonella* serotypes ; *Salmonella*

Typhimurium and *Salmonella* Enteritidis which are the most common serotypes in the United States and those serotypes are more prevalence in the summer than winter.

According to CDC report, approximately 42,000 cases of salmonellosis are recorded in the United States annually. The actual number of infections may be excess this number because many cases are not diagnosed or not reported (CDC 2013). The symptoms associated with *Salmonella* infection are diarrhea, fever, and abdominal cramps that begin 12 to 72 hours after ingesting the food contaminated with *Salmonella* and lasts 4 to 7 days. Most people recover without treatments. However, some people may need to be hospitalized due to severe diarrhea. In rare conditions, the bacteria can move to the bloodstream from intestines causing severe illness or death if not treated with antibiotics. The mode of transmission is the fecal-oral route. Therefore, *Salmonella* can be transmitted from food, water that has been contaminated with fecal containing bacteria or from contact with infected animals (CDC 2013).

Low Aw products and Salmonella outbreaks

The water activity defined as the ratio of the water vapor pressure of the food to the water vapor pressure of pure water (Jay 2005). Water activity in food refers to the amount of water available for chemical or microbiological reactions; this water can support the growth of microorganisms including bacteria, yeasts and molds. Therefore, microorganisms cannot grow in low or absence of unbound water in food (Christian 1963). In general for many vegetative pathogens including *Salmonella* spp., low water activity is obstructive to their growth. However, several studies indicated that *Slamonella* may survive in dry foods and feeds for a long time (Hirmatsu, R. et al. 2005,

Janning, B. et al. 1994, Juven, B. J. et al. 1984).Hirmatsu, R. et al.(2005) determined the ability of *Salmonella* spp. to survive under low Aw condition ranging between 0.5 to 0.6 on paper disk while *Escherichia coli*, didn't survive under the same condition. Janning et al. (1994) determined that *Salmonella* was more resistant to desiccation under the experimental condition than were *Enterobacter Cloacae* and *Escherichia coli*. This author evaluated the survival of 18 bacteria strains, including 9 strains (*Salmonella*) under dry conditions (Aw of 0.2) at 22 °C. According to his study, *Salmonella* strains remained stable for long time; 248 to 1351 days were needed to achieve an 1-log reduction.

Almonds

Two outbreaks of salmonellosis have been linked to consumption of raw almonds in 2001-2004 in USA (Chan et al., 2002; Isaacs et al., 2005; CDPH, 2002) and Canada (Keady et al. 2004; CDPH 2004). In USA 168 cases were reported and in 2004 47 cases were reported in Canada. In 2006 an outbreak of salmonellosis was reported in Sweden that linked to consumption of whole raw almonds. A number of 15 cases of *Salmonella* Enteritidis NST 3+ were recorded with *Salmonella* Enteritidis NST 3+. The results from case control showed that eating almonds were a risk factor for infection with *Salmonella* Enteritidis NST3+ (Ledet et al. 2007). In 2012 another outbreak of 27 cases of salmonellisis has been associated with consumption of raw almonds in Australia (Whitworth et al. 2012). According to this author a potential transmission is still unknown. As mentioned above, two outbreaks of *Salmonella* infection were linked to consumption of raw almonds in 2001 and 2003. However, no outbreaks of *Salmonella* infection were reported that associated with the consumption of almonds before 2001.

The reason why there were no outbreaks before 2001 may be a matter of deeper analysis. On one side, non-reported outbreaks of *Salmonella* infection with almonds in past could have but cases might not have been reported, or the consumption of almonds could not be established as the source of infections. On the other side, it is possible that environmental conditions might have prevailed for the time when the outbreaks occurred, favoring an increase in the hazard populations and subsequent dissemination. According to many studies, almonds may be contaminated during harvesting and post harvesting from the environment (Danyluk et al. 2008b, Reil et al. 1996). Therefore, scientific evaluation of environmental conditions is needed to compare the differences in environmental changes in recent years.

Chocolate and coconut

Chocolate is one of products that have very low moisture content. The moisture in this product is less than 8%. However, this product has contributed to many *Salmonella* infection outbreaks (Craven et al. 1975; Gill et al. 1983; Hockin et al. 1989; Kapperud et al. 1990; Werber et al. 2005). In 1973 and 1974, 80 cases of *Salmonella* infection were linked to consumption of Chocolate in USA, and 39 other cases were reported in the same period in Canada. A strain of *Salmonella* that isolated from several samples of chocolate and caused the outbreak was *Salmonella* Eastbourne (Craven et al. 1975). In 1982 another outbreak of *Salmonella* infection was linked to chocolate in England and 245 cases were reported with *Salmonella* Napoli infection. According to the investigation to this outbreak two types of chocolate were the vehicle of this infection (Gill et al. 1983). Between 1985- 1986 an outbreak of *Salmonella* Nima enterocolitis

was associated with consumption of gold-foil wrapped chocolate coins and result in 92 infection cases in Canada and 4 cases in the United States (Hockin et al. 1989). In 1987 an outbreak of *Salmonella* Typhimurium infection occurred in Norway and Finland and caused 349 infection cases in Norway and 12 cases in Finland (Kapperud et al. 1990). In 2001-2002 a total of 439 cases of *Salmonella* Oranienburg infections were recorded in Germany due to the consumption of dark chocolate (Werber et al. 2005). Despite reports from the investigation that in most of outbreaks only a very low levels of *Salmonella* or contamination were found, *Salmonella* still could cause the illness and infection (GMA 2009). The studies suggested that high fat content of chocolates may support *Salmonella* to colonize in gastrointestinal and cause infection even with small number of cells in products (Craven et al., 1975; D'Aoust, 1977). Ultimately, in 1953 an outbreak of *Salmonella enterica typhi*, *Salmonella* Senftenberg and possibly others have been implicated with desiccated coconut in Australia (Wilson and Mackenzie 1955).

Peanut

Peanut has been contributed to several epidemical outbreaks of different strains of *Salmonella* in different years and places. In 1994-1995 an outbreak of *Salmonella* Agona PT 15 was associated with flavoured peanut ready to eat kosher savoury snack in United Kingdom, Israel, USA and Canada. A number of 78 cases were reported, and cases of infection with group B *Salmonella* increased from 60% to 80% in children under 5 years old. *Salmonella* Agona phage type 15 was isolated from samples of this snack; this outbreak of *Salmonella* Agona was caused by the contamination of a snack produced in Israel and caused an international outbreak (Killelea et al. 1996, Shohat et

al. 1996, Threlfall et al. 1996). In same year, another outbreak also was linked to consumption of peanut butter in 1996 in Australia. A total of 15 persons were reported with infection of *Salmonella* enterica serovar Mbandaka in South Australia , thirteen out of 15 were consumed the brand of peanut butter that produced by the same company. *Salmonella* Mbandaka was isolated from 3 jars of peanut butter from case households and other three jars from retail outlets. (Scheil et al. 1998). Additionally, in 2001 an outbreak of *Salmonella* Stanley and *Salmonella* Newport was linked to consumption of flavored or roasted in – shell peanut in Australia, Canada, and United Kingdom. In total, 97 cases of *Salmonella* Stanley and 12 cases of *Salmonella* Newport infection were found. (Kirk et al. 2004). A widespread outbreak in 2006-2007 associated with boiled peanut butter in 47 states in USA and a number of 628 cases were reported with *Salmonella* Tennessee. The outbreak was associated with two brand of peanut butter(Peter Pan or Great Value), and the outbreak strain of *Salmonella* Tennessee subsequently was isolated from several opened and unopened jars of Peter Pan and Great Value peanut butter. The outbreak has investigating to determine the mechanism of contamination. However, the source of the peanut butter contamination is unknown (CDC 2007a ; 2007b) . It thought that peanuts can become contaminated with *Salmonellae* during growth, harvest, or storage, and the *Salmonella* are able to survive high temperatures in a high-fat, low-water--activity environment (Mattick 2001). The outbreak demonstrated that processed food can become contaminated even with applying a heat-treatment step, effective preventive controls in food-processing plants is needed to prevent contamination (CDC 2007a). The outbreak of *Salmonella*

Typhimurium was linked to the consumption of peanut butter in 2008-2009 in 46 states in USA and one case in Canada. A total of 714 cases were infected during this outbreak and *Salmonella* Typhimurium was isolated from King Nut creamy peanut butter sample, also isolated from other peanut butter products such as peanut butter crackers. According to Epidemiologic and laboratory the peanut butter and peanut paste produced at one plant are the source of the outbreak. A control measures as a response to this outbreak were recalled all and stopped the production of peanut butter and paste peanut related to the company with brands associated with outbreak, and treatments include dry- and oil-roasted peanuts, granulated peanuts, and peanut meal (CDC 2009a and 2009b, Cavallaro et al. 2011). Finally, in 2012 an outbreak of *Salmonella* Bredeney was linked to consumption of peanut butter in USA included 19 states, a total of 42 cases were infected with the outbreak strain *Salmonella* Bredeney (CDC 2012).

Pine nut

In 2011 an outbreak strain of *Salmonella* Enteritidis was linked to consumption of whole, bulk Turkish pine nut in USA (MD, NY, NJ, PA, VA), and *Salmonella* Enteritidis was isolated from the samples, and 43 cases were reported from 5 states. According to CDC investigation, among 30 infected persons, 19 (63%) were associated with consuming Turkish pine nuts or products containing these pine nuts in the week before their illness began. (CDC 2011b).

Sesame seed or helva

In 2001 an outbreak strain of *Salmonella* Typhimurium DT 104 in Australia, Sweden, Norway, United Kingdom, and Germany was attributed to contaminated sesame seed or halva. In Australia and Sweden 17 cases were reported, 27 cases were reported in Norway and United Kingdom, and 18 cases were reported in Germany, In Sweden *Salmonella* Typhimurium DT 104 has been isolated from five jars of helva that flavored with pistachio and cocoa and matched with patient feces samples (O'Grady 2001, deLong 2001, Brockmann 2001, Little 2001, Aavitsland et al.2001). The *Salmonella* Typhimurium DT 104 has been responsible for several infections in European countries. Because it is the strain that is resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines , it called multiresistant, it is known to be the most prevalent multiresistant phage type (Brockmann 2001, Aavitsland et al.2001).

Tahini and helva

Three outbreaks of *Salmonella* Montevideo has been associated with consumption of Tahini. In 2002-2003 in Australia and New Zealand and other outbreak of the same strain was linked to consumption of helve in 2003 in Australia. In 2002 a total of 55 cases were reported, and in 2003, thirteen cases were reported with infection of *Salmonella* Montevideo (Unicomb et al. 2005).

Control of Salmonella in low moisture food

In general, most of the microorganisms including pathogens cannot grow in low water activity or low moisture food products because low water activity is obstructive to

their growth. However, many outbreaks of *Salmonella* infection have been associated with the consumption of low water activity food products such as almond, peanut butter, chocolate, infant formula (CDC 2004, 2007, 2009, Chan et al., 2002; Isaacs et al., 2005, Killelea et al. 1996, Kapperud et al. 1990). According to the investigations of the factors of these outbreaks, the potential sources of the contamination of the low moisture products is cross contamination which is the transfer of bacteria from contaminated surface or environment to other non contaminated surface, poor sanitation practices also play a major role in increasing the risk of contamination (GMA 2009, Scott et al. 2009). To control or reduce the risk of *Salmonella* in low water activity products, good manufacturing practices have to be the priority of the industries and food processors, and effective controlling measures should be applied (GMA 2009, Scott et al. 2009). After *Salmonella* outbreaks that implicated with consumption of raw almonds, thermal and non-thermal pasteurization interventions were proposed to eliminate *Salmonella* in almonds. Most current pasteurization methods rely on heat as an intervention including blanching and oil roasting (Du et al. (2010); the thermal inactivation of *Salmonella* Enteritidis PT 30 in almonds is influenced by water activity. The evaluation of the impact of water activity was implemented by Villa-Rojas et al. (2012). According to this study the decimal reduction time decreases with increasing water activity. Two studies have shown that heat resistant *Salmonella* in low Aw foods can survive the current thermal pasteurization treatment (Mattick et al. 2000; Pan et al. 2012). Mattick et al. (2000) found that heat resistant *Salmonella* will increase in foods containing low Aw ingredients within moist environments. The authors also found that exposure of

Salmonella spp. to low A_w could reduce the effectiveness of any subsequent heat processing, and *Salmonella* could survive on the almonds that reached the consumers and cause illness (Mattick et al. 2000; Pan et al. 2012). Thus, raw almonds in the USA may pose a relatively high risk to the consumers. A risk assessment was proposed by Danyluk et al. (2006) where Monte Carlo simulations were conducted to model the effect of factors such as storage time, pre - process, post process and reduction level of *Salmonella* during storage. The heat resistance of *Salmonella* is increased at low water activity compared with in moist environments (Mattick et al. 2000). Non-thermal pasteurization process may be an effective treatment as well. However, some non-thermal treatments may minimally affect the sensory taste and quality of the food products. Obviously, not every non- thermal treatment will satisfy the consumers because consumers need the almonds with good sensory and quality besides the safety (Pan et al. 2012), among non-thermal treatments electron beam irradiation has been demonstrated to be an effective treatment of eliminating and inactivating both pathogenic and spoilage microorganisms in the food products (Dickson 2001).

Good agriculture practices

Controlling *Salmonella* in low water activity food is more difficult than high water activity products. Therefore, to control *Salmonella* in low A_w food, the effective control measures should be performed and focus particularly on preventing contamination. Good Agriculture practices is one of the most efficient control measures to prevent contamination, this approach is based on efficient cleaning and sanitation procedures and hygienic design in the food processing and environment(Beuchat et al.

2013). According to the study by Craven et al. (1975) inadequate separation between clean and unclean environment may increase cross contamination, and this study suggested that controlling airborne diffusion of the dust reducing cross contamination by *Samonella* Eastbourne. Therefore, it crucial for low food processors and industries to apply good agriculture practices (GAPs) which are include sets of recommendation that help to reduce the risk of *Salmonella* in these communities and improve the quality and safety of products. In order for industry and processors to have a reference for good agriculture practices, in 2009 ABC published the guidance document for the control of *Salmonella* in low moisture products and stressed that these products at risk being contaminated with biological, physical, and chemical hazards, biological hazards include *Salmonella*, *E. coli* and other Shiga toxin. Physical hazards include stones, glass and metal. And chemical hazards include pesticides, food allergens and aflatoxins (ABC 2009). The guidance involved recommendations that is applicable to various products such as peanut butter, cereals, dry protein products (such as dried dairy products, soy protein, rice protein), confections (such as chocolate), snacks (such as corn chips), spices, animal feeds (both ingredients and finished products).

According to this document, in order for industries and processors to minimize a risk of *Salmonella*, seven principles should be applied in low moisture foods which are: prevent ingress or spread of *Salmonella* in the processing facility, enhance the stringency of hygiene practices and controls in the Primary *Salmonella* Control Area, Apply hygienic design principles to building and equipment design, prevent or minimize growth of *Salmonella* within the facility, establish a raw materials/ingredients control

program, validate control measures to inactivate *Salmonella*, and establish procedures for verification of *Salmonella* controls and corrective actions (GMA 2009).

Mechanisms for Salmonella survival and heat resistance

After several outbreaks of *Salmonella* illness that have been implicated with low moisture products, many studies have investigated the behavior of *Salmonella*, and have shown that *Salmonella* can survive for a long period of time in foods and feeds (Juven et al., 1984; Janning et al., 1994; Hiramatsu et al., 2005). Another study suggested that when *Salmonella* is exposed to higher or lower A_w , the organism may induce adaptation to dry environment. The adaptation of *Salmonella* to dry environment depends on A_w that *Salmonella* exposed to whether the exposure levels high or low, and this adaptation may result in physico-chemical changes in the organism due to environmental pressure, leading to formation of a protective biofilm, and/or entry into a dormant state (De Rezende et al. 2001). Therefore, according to this study, the organism either enter a viable but nonculturable (VBNC) state or forming biofilm in order to survive inadequate conditions. But it is no clear whether *Salmonella* cell can form biofilm in high or low A_w . However, De Rezende et al. (2001) demonstrated the ability of *Salmonella* Typhimurium DT104 to extensive filamentation after exposure to low A_w . However, both mechanisms of survival of *Salmonella* result in heat resistant of *Salmonella* by inducing a number of stress response that result in heat resistance and extent their survival in low A_w foods. A study by Abee et al. (1999) showed that the adaptation to the stress may enhance the survival of *Salmonella* in a variety conditions. This author examined adaptation of *Salmonella* Typhimurium to osmotic stress, and found that

microorganism adapt to environment by changing nutrients or to response to the stresses in minimal processing, include; controlling metabolic change by genetic switches by modification of sigma (σ) factors bind to core RNA that result in heat-shock response, the chemotactic response, sporulation, and general stress response (Abee et al. 1999, GMA 2009). In conclusion, the stress response of *Salmonella* generates a resistance that enable *Salmonella* to survive and persist in extreme environmental conditions (e.g., soil and water), food processing and handling, and environments and the response generated may provide more cross- resistance to a variety of hard conditions (Spector and William 2011). To eliminate or control *Salmonella* in low A_w foods, the organism should subject to effective controlling measures or the combination of more than one treatment due to the ability of *Salmonella* to survive adverse conditions and to generate heat resistance.

Thermal pasteurization of almond

Two outbreaks of salmonellosis have been implicated to consumption of raw almonds, In the result of these outbreaks, Almond Board of Californian under supervision of USDA has implemented an action plan required all almonds processors to sterilize their almonds before reaching consumers (ABC 2007). According to this action plan, inactivation steps should be applied to the almonds to achieve at least 4-log reduction of *Salmonella* in almond. Most controlling measures include heat treatments as an intervention to control *Salmonella* in almonds. Heat treatment or pasteurization process defined as a mild heat that aimed to inactivate pathogenic and spoilage organisms (Da Silva and Gibbs 2009). The common industry pasteurization processes include blanching and oil roasting. The National Advisory Committee on

Microbiological Criteria for Foods (2006) defined pasteurization as “Any process, treatment, or combination thereof, that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage” (Pan 2012). However, the temperature resulting from the heat treatments may affect negatively the quality of almonds including the vitamins, essential nutrients, and flavors (pan 2012).

Blanching

Blanching is one of the pasteurization methods approved by FDA (ABC 2009). This treatment is applied to almonds to eliminate or reduce the populations of *Salmonella* on almond surfaces. Commercially blanching is used by almond processors to remove almond skins. The time and temperature used in this process depend on the almond variety, the moisture content, and the type of blanched product (whole, whole and broken, pieces, cutting inputs, etc.) (ABC 2007b). Blanching consists of two steps; scalding and drying, in the scalding step almonds are exposed to hot water, and temperature of hot water is controlled by a thermocouple attached to the immersion section. The temperature of the scalding water may be affected by many factors such as equipment temperature setting, almonds temperature at beginning, and the temperature of the hot water temperature may not reaching the required temperature, etc. In such conditions, validation testing is required to check the effects of these factors. For blanching to be effective, at least 4-log reductions of *Salmonella* should be achieved, blanching needs to heat for 2 min to achieve this requirement (ABC 2007b). Harris et al. (2011) examined the efficacy of blanching process by exposing the almonds inoculated

with *Salmonella* Enteritidis PT 30, *Salmonella* Senftenberg 775W and *Enterococcus faecalis* to hot water. In this study almonds were heated for 2 min at 60, 70, 80 and 88 °C temperatures. According to this study, D-value for *S. Enteritidis* PT 30 after exposing to hot water at 60, 70, 80 were 2.6, 1.2, 0.75, and 0.39 min respectively , and D-value for *Salmonella* Senftenberg 775W and *E. faecalis* at 88 °C were 0.37 and 0.36 min, respectively. This study demonstrated that neither *Salmonella* serovar was recovered by enrichment of 1-log samples after almonds inoculated at 5 logs CFU/g were heated at 88 °C for 2 min. This study confirmed the efficacy of blanching as intervention treatment for almonds heating for 2 min.

Oil roasting

Oil roasting is a process that is used by the industry to roast almonds for the purpose of obtaining crunchy and roasted flavor almonds. This process commercially is performed by pre-heating the oil tank. The roasting process starts when the almond kernels are carried by a continuous conveyor through the tank. Then, the almond kernels are submerged in the hot oil. The time of roasting is controlled by the conveyor speed dial setting, and the temperature of oil is controlled by a thermocouple that is submerged in the oil tank. In commercial roasting process, the roasting combined with salting and flavoring processes. The temperature- time treatments required for commercial oil roasting range between 137 and 121° C for 3 to 15 min (ABC 2007b). Oil roasting is used as one of the pasteurization methods to control *Salmonella* in almonds and approved by ABC . Du et al. (2010) evaluated oil roasting by inoculating almonds with *Salmonella* Enteritidis PT 30 or *Salmonella* Senftenberg 775W and heating in oil at 116,

121, and 127 °C respectively. According to this report, rapid reductions of 2.9, 3.0, or 3.6 log CFU/g for *Salmonella* Enteritidis PT 30 were observed after 30 s of exposure to oil at 116, 121, and 127 °C and similar results were obtained for *Salmonella* Senftenberg 775W. For oil roasting to achieve 4 and 5 log reduction of *Salmonella*, 0.74 and 1.3 min at 127 °C respectively was required. This study showed that neither *Salmonella* serovar could be recovered after 1.5 min of oil roasting treatment. This indicated that for industry to achieve 5-log reductions of *Salmonella* in almonds by the oil roasting intervention, less than 1.5 min would be required.

Other thermal treatments

As mentioned before blanching and oil roasting are the most common thermal interventions to control *Salmonella* in almonds. Since the objective of thermal treatment is to inactivate pathogenic and spoilage microorganisms, researchers have evaluated different thermal treatments other than traditional blanching and oil roasting, either alone or combined two or more treatments to achieve more reduction and reduce time and energy required. Jeong et al. (2009) evaluated moist-air heating on the surface of inoculated almonds with *Salmonella* Enteritidis PT30 processed in a computer-controlled laboratory-scale convection oven. These authors found that using moist-air decreased heat resistance of *Salmonella* and the time required to achieve 5-log reduction of *Salmonella*. By applying steam on the surface of cold almond, this process increased water activity and reduction of *Salmonella* on the almond surface. Then evaporation started after the temperature increased on surface and condensation of the steam stopped. According to this study, increasing the moisture on the surface of almonds helped to

reduce the time required to inactivation by decreasing heat resistance of *Salmonella*. Bingol et al (2011) investigated the efficacy of an infrared (IR) pasteurization process on the reduction of *Salmonella* on almonds . In this process almonds were heated to 100, 110, and 120 °C with infrared (IR) pasteurization and cooled at ambient temperature with holding temperature of 70, 80, and 90 °C for different times before almonds were transferred to a holding device. According to this study, holding temperature at 90 °C for 10-15 min reduced the population by 5-log , whereas holding at 80 °C for 22 min reduced the population by 4-log cycles. The study also suggested that this process did not significantly affect the overall quality of almonds. Chang et al. (2010) evaluated the feasibility of steam pasteurization for reducing *Salmonella* on almonds. In this study inoculated almonds were dried overnight and steam treatments were applied through a pilot-sized vertical pasteurization machine for 5, 15, 25, 35, 45, 55 and 65s. According to this study, reduction of *Salmonella* increased with steam exposure time, and suggested that 25s was sufficient to achieve 5-log reduction of *Salmonella* with no noticeable effect on the almond quality. Lee et al. (2006) also examined the efficacy of steam pasteurization on the reduction of *Salmonella* on almonds, in this study, the same almond variety and the same *Salmonella* strain was used as in Chang et al. (2010). However, unlike the previous study, these authors observed that 5-log reduction of *Salmonella* could not be achieved even after 35s of steam treatment, and they agreed with Chang that exposure to steam for a long time affected the quality and changed in the almond texture. Bari et al. (2009) examined the effectiveness of combining of different treatments on the reduction of *Salmonella* on the almonds. In this study,

sanitizers (strong or mild electrolyzed water, ozonated water, and distilled water), dry heat treatment, and hot water treatment and catalytic infrared (IR) heat treatment were used to inactivate *Salmonella* on raw almonds. This study indicated that more reduction, even more than 5-log cycles could be achieved by combining treatments. Yang et al. (2010) also assessed the effectiveness of the sequential infrared and hot air roasting on the reduction of *Salmonella*. This study showed that by combining the hot air roasting with sequential infrared allowed a shorter roasting time, improving food safety and suggested that a new method of oil roasting could be easily be implemented by the almond industry.

Non-thermal pasteurization

Most thermal processes rely on heat as an intervention to inactivate *Salmonella* in almonds. However, heat treatments may be ineffective against heat resistant strains of *Salmonella*. This pathogen; can survive and persist in almonds even after heat treatments. Non-thermal technologies may be an alternative to inactivate heat resistance strains of *Salmonella* in almonds and other low water activity food products. The objective of non- thermal treatments is to inactivate pathogenic microorganism with minimum effects on quality and sensory losses of the products (Barbosa-Canovas et al. 1998). Among non- thermal treatments that were tested on food are high hydrostatic pressure, pulsed electric fields, ultrasound, ultraviolet light, cold plasma, irradiation, pulsed light, dense phase carbon dioxide and shock waves, only high hydrostatic pressure and irradiation have been approved and used in commercial settings on several foods. The other non-thermal approaches are still under research. (Bermúdez et al.

2012). Goodridge et al. (2006) evaluated the efficacy of high hydrostatic pressure on inoculated almonds with two strains of *Salmonella* Enteritidis PT30 and FDA. In this study, almonds inoculated with *Salmonella* Enteritidis were placed in vacuum-sealed bags and were pressurized at continuous 50,000, 60,000 and 70,000 psi at 25, 50, 55 °C for 5 and 10min and discontinuous (oscillatory) six cycles at 60,000 psi at 25, 50, 55 °C for 20 s. The continuous pressurization resulted in less than one log reduction at 25 °C for 5 min. However, discontinuous process at 60,000 psi and 50 °C for 20 s resulted in more than one log reduction. According to this study, low water activity was one of the factors that had a protective effect against the treatment. In this study, the antimicrobial effect of HHP increased by suspending the inoculated almonds in 0.1% peptone solutions, resulting in reductions of 2.04- and 8.16-log cycles in *Salmonella* Enteritidis depending on pressure, time and temperature of exposure. This study confirmed that HHP is more effective with foods with higher water activities.

Non-thermal plasma (NTP) or cold plasma is a novel non-thermal food processing technology (Niemira 2012). It consists of a neutral ionized gas that comprises highly reactive species including, positive ions, negative ions, free radicals, electrons, excited or non-excited molecules and photons at room temperature (Afshari et al. 2012). Niemira (2012) examined the efficacy of cold plasma intervention on dry almonds inoculated with *Salmonella* and *E. coli* O157:H7. In this study, inoculated almonds were subjected to cold for 10 s, or 20 s, the distance from the emitter was 2, 4, or 6 cm, and feed gas was dry air or nitrogen. The reduction was 1.34 log of *E.coli* O157:H7 after 20 s with distance of 6 cm. According to Niemira (2012), reduction of pathogens using NTP

is due to UV light and reactive chemical products of cold plasma ionization process. This study showed that reductions greater than 5 log cycles can be obtained for pathogens such as *Salmonella*, *E. coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* with effective treatment times. This technology also was proposed by Deng et al. (2007) as an alternative non-thermal technology to reduce *E. coli* on unshelled almonds. These authors obtained 1.0 and 4.1-log reductions in *E. coli* were obtained with electrodes set to 16 and 25 kV in 30 s. The study also suggested that with increasing the frequency of application at a constant voltage resulted in a greater reduction rate. For instance, a 5.60-log reduction could be achieved at 2,500 Hz frequency. However, the application of NTP technology is limited due to unknown effects on nutritional and sensory qualities on almonds (Niemira 2012).

Propylene oxide (PPO) is considered one of the non-thermal treatments that is commercially used in US as fumigant to inactivate bacteria, mold, and yeasts. It is approved by FDA for pasteurizing of almonds. A reduction of 5-log cycles in *Salmonella* Enteritidis on almonds can be achieved by using PPO. Additionally PPO has showed no effects on the sensory quality on almonds. However, the Environmental Protection Agency (EPA) mandated the PPO exposure time not exceed 4 h and the residual limit of 300 ppm. The effectiveness of PPO on the reduction of *Salmonella* Enteritidis PT 30 on inoculated almonds has been evaluated by Danyluk et al. (2005), in this study, a dry inoculated almonds were placed in bags designed for gaseous sterilization and placed in the center of 22.7-kg boxes of warmed almonds and then warmed to temperature 23 to 34°C and treated with PPO (0.5 kg/m³ for 4 h) and held for

0 or 2 days at 38 to 43°C , then almonds were stored for 2 to 5 days at 15 to 18°C. the reduction of *Salmonella* were >5.0 log after 5 days of treatment and no significant reduction was obtained when almonds were held at 38 to 43°C after PPO. The residues were <300 ppm during post- PPO storage after there were >400 PPO after almonds were removed from the chamber. This study demonstrated that PPO was an effective intervention to inactivate *Salmonella* Enteritidis PT 30 on almonds as long as PPO exposure time and the residual limit are maintained.

Electron beam irradiation

Electron beam irradiation has been approved by FDA is for reducing pathogenic microorganisms in certain food products (Pauli and Clyde A. T. 1986). Electron beam irradiation utilizes high speed releasing energy to target microbial DNA and causes damage or destroys pathogens in food products by the use of high-energy electrons (Murano 2010). Electron beam irradiation is considered a cold pasteurization process because it does not raise the temperature of the food being processed. As with other source of energy for food irradiation, the food does not come into contact with the radiation source during electron beam irradiation. Therefore, this process does not induce radioactivity in the food when using electron beams up to 10 MeV, as regulated by the International Atomic Energy Agency (IAEA). Therefore, the foods that are subjected to electron beam irradiation are safe (Farkas 2004). Thus, irradiation makes food safer and extends the shelf life of foods, and nutrient losses are small and often similar to or less than, the losses caused by other pasteurization methods. Food irradiation was evaluated in 1980 by an expert committee representing the World Health

Organization (WHO), the United Nation Food and Agriculture Organization (FAO), and IAEA. This committee concluded that any irradiated foods were safe to consume when using up to 10 kilograys (kGy). Later, the same committee concluded that foods irradiated at any dose were safe (Murano 2010). However, two concerns should be taken into account when using electron beam irradiation on almonds: sensory quality and consumer acceptance. Although the objective of using irradiation is eliminating microorganisms, the sensory quality of the final products is also important (Pan et al. 2012). The nutritional and sensory quality of irradiated foods depend on a number of factors such as irradiation dose, temperature, food composition, and the presence or absence of oxygen. However, those challenges can be reduced by controlling the irradiation dose, or through irradiation of food in an oxygen free atmosphere (Murano 1995). In addition, the consumer's knowledge about the benefits of using electron beam irradiation on food products is still a great challenge and may lead to a negative effect on the ability of the industry to apply this process, due to how consumers rate the irradiated products. The mechanism of inactivation of irradiation occurs either by direct or indirect effects on microorganisms. Direct effect occurs by collision between the radiation energy and the genetic material through damaging a critical element in microbial cell, especially genetic material. This damage hinders organisms from multiplication and terminates most cell activities. The indirect effect occurs by radiation ionizing of water molecules, which leads to bacterial death by interacting free radical with the bacterial cell membrane to change or damage their structure (Dickson 2001; Murano 2010). According to Prakash et al. (2010), using electron beam irradiation on almonds was

effective against *Salmonella* serotype PT 30 and a 4-log reduction can be achieved, but the irradiation dose needed to meet this mandatory treatment rendered the almonds unacceptable to consumers. In summary, among all the above-proposed pasteurization technologies, only PPO, hot water, steam, and hot oil processing have been approved by FDA as commercial interventions for almonds. The remaining other technologies are still under research and require to be evaluated by the ABC Technical Expert Review Panel for their feasibility to achieve a minimum 4-log reduction of *Salmonella*. In addition, the proposed technologies have to preserve almonds nutritional characteristics and the sensory quality (Pan et al. 2012).

CHAPTER II

MATERIALS AND METHODS

Materials and methods

Almond acquisition and storage

Unpasteurized Nonpareil almonds were obtained from a supplier in California. The water activity was taken upon almond arrival by using an AquaLab water activity meter (Aqua Lab Series 3, Decagon Devices, Inc.) in order to have a baseline water activity. The almonds then were stored under refrigeration until the start of the experiment.

Bacterial cultures

Salmonella Enteritidis PT 30 ATCC 1045 and *Salmonella* enterica serovar Typhimurium strain LT2 were used in this study. Both strains were obtained from the bacterial culture collection of the Food Microbiology Laboratory at Texas A&M University in College Station (TAMU). The strains then were activated by transferring one cryopellet into tryptic soy broth (TSB; Difco, Spark, MD) followed by incubation at 35 °C for 24 h. the stains then were streaked on tryptic soy agar slants (TSA; Difco) and were stored at 25 °C until needed.

In order to obtain traceable derivatives of *Salmonella* strain, a spontaneous variant of both *Salmonella* Enteritidis PT 30 and *Salmonella* Typhimurium LT2 expressing resistance to rifampicin (rif+) was selected. The procedure for obtaining the

rif⁺ derivative is a modification of the method describe by Kaspar and Tamplin (1993). In this modified procedure, the inoculum was prepared by transferring the growth of *Salmonella* strain from TSA slant into 9 ml tryptic soy broth (TSB; Difco, Spark, MD) followed by incubation at 35 °C for 24 h. After 24 h the strain was aseptically streaked on tryptic soy agar (TSA; Difco) and incubation (Gallenkam/Sanyo; San Diego, CA) for 24 h at 35 °C. After 24 h the single pure colony was taken from the plate and was aseptically streaked on tryptic soy agar (TSB; Difco) supplemented with 100 µg rifampicin (Sigma Chemical Co.)(TSA+ Rif) followed by incubation at 35 °C for 24 h. Then a single pure colony was streaked on TSA slant and used as working culture.

Preliminary study

The effect of accelerated and conventional drying on the moisture of almonds, A_w measurements was conducted on almonds after inoculating, and at various times during drying. Although not a true A_w value of almonds, A_w measurement can provide an accurate reading of the amount of free water on the surface of the almonds as a result of the inoculation and further drying. Prior to the start of the experiment, the water activity of almonds was measured using the AquaLab water activity meter to obtain the baseline level. Then, two samples of 400 g of almonds each were placed in an individual polyethylene bag(36 cm x 40 cm, Pactiv Corporation, Lake Forest, IL) and inoculated with *Salmonella* Enteritidis PT30 , mixed thoroughly by hand shaking by inverting the bag up and down 50 times for 2 min. A sample of 20 ± 2 g of almonds were taken immediately after inoculation for water activity measurement, the sample was ground using a food processor (Black and Decker; Baltimore, MD) and the ground

almonds were transferred to individual disposable containers (Decagon Devices, Inc., Pullman, WA) and were covered with airtight lids to prevent any gain or loss of moisture. Then, the AquaLab meter was calibrated according to the device standard by using two identical samples of distilled water. Then, the containers were placed in the water activity meter, one at a time. Water activity of almonds was determined following inoculation and after 3, 6, 9, 12, and 24 h. The same procedures were used for both almonds with accelerated drying and almonds with conventional drying in the same time to determine the differences in water activity after 24 h. The procedure for accelerated drying is described below. This procedure was replicated three times in three different days.

For microbiological analysis, different media were used in order to compare the growth characteristic of *Salmonella* in different media. In this study, tryptic soy agar (TSA ; Difco) was used as a standard. The TSA was supplemented with rifampicin (Sigma Chemical Co.) to have a selective medium for the Rif⁺ microorganisms used in the study. TSA supplemented with 2.5% NaCl was used to detect any possible injury caused by the drying procedures. For microbiological analysis, the samples of 20±2 g were taken after inoculation, then every 3 h until 24h from inoculation. The inoculated almonds were placed into a stomach bag and were blended by Stomacher Lab Blender 400 (Model BA6021, A. J. Seward, London, UK) for 1 min, serial 10-fold dilution of each sample was prepared in sterile 0.1 % peptone water and was surface spread onto TSA + Rif. Plates were incubated at 35 °C for 24 h, and the colonies were enumerated using a Quebec colony counter.

Accelerated drying

Since the inoculation of almonds was expected to modify the water content on the surface of the almonds, a previously developed method (Cuervo 2011) was followed for restoring the almond moisture to the original values. A sample of 400 g of almonds was placed in polyethylene bag (36 cm x 40 cm, Pactiv Corporation, Lake Forest, IL) and inoculated with *Salmonella* Enteritidis PT30 or *Salmonella* Typhimurium LT2 as described below. Inoculated almonds then were placed onto a surface of 2 sheets of absorbent paper (9.125x10.25 inch; Tork, Hand Towel Single fold White) extended inside a sterile plastic tub. A sample of 20 ± 2 g of almonds was taken immediately after inoculation for water activity measurement. Almonds were then placed in an incubator at 35°C for accelerated drying for 12 h. Then, the almonds were taken and placed in a glass desiccator with a glass bowl containing a saturated solution of NaBr (Mallinckrodt Baker) to prevent further changes in the A_w . The desiccator with almonds was maintained at $23 \pm 2^\circ\text{C}$ for an additional 12h. Then another sample of 20 ± 2 g of almonds was taken for water activity measurement.

This accelerated drying procedure was compared with the procedure described by Danyluk et al. (2005), which consisted of, placing another inoculated 400 g almond sample onto the surface of a stack with 2 sheets of absorbent paper (9.125x10.25 inch; Tork, Hand Towel Single fold White) extending inside a sterile plastic tub, which was loosely covered with aluminum foil. The almonds then were allowed to dry for 24 h at $23 \pm 2^\circ\text{C}$.

The moisture content of almonds also was determined by calculating the % moisture for almond kernels. For measuring the moisture, a vacuum oven was used. The mass of the almonds before and after drying was determined as follows:

The weight of a small container (cardboard container) was taken, then 2-3 g of almonds (3-4 almonds) were placed in the container and the weight was determined using a sensitive weight scale, the sample with container were placed onto vacuum oven for 16 h at 105-110 °C. After 16 h of drying, the weight of sample with container was taken.

Then the moisture content was measured using the following formula (Trautmann N. et al. 2001).

$$M_n = ((W_w - W_d) / W_w) \times 100$$

in which: M_n = moisture content (%) of sample

W_w = wet weight of the sample, and

W_d = weight of the sample after drying

Inoculum preparation

The rif+ *Salmonella* Enteritidis PT30 or the rif+ *Salmonella* Typhimurium LT2 were individually transferred from TSA slant into 9 ml TSB and incubated at 35 °C for 24 h in order to obtain a bacterial lawn. These cultures were then transferred into centrifuge tubes (sterile conical tubes) and were washed by centrifugation at 3500 x g for 15 min; the resulting pellets were resuspended in 25 ml of phosphate buffer saline (PBS). In order to obtain information about the inoculum concentrations, serial 10-fold dilutions of this suspension were prepared in sterile 0.1 % peptone water (PW; Difco),

spread plated onto TSA+Rif and incubated (Gallenkam/Sanyo; San Diego, CA) at 35 °C for 24 h before colony enumeration by a Quebec colony counter.

Inoculation of almonds

Prior to inoculation, the water activity of almonds was determined using a water activity meter (Aqua Lab Series 3, Decagon Devices, Inc.). Two sets of 400- g almond samples were prepared and placed in individual polyethylene bags (36 cm x 40 cm, Pactiv Corporation, Lake Forest, IL). To inoculate, 25 ml of the 24 h prepared suspension were added to each bag containing 400 g of almonds. Almonds and inoculum were mixed by hand shaking the bags for 2 min vigorously up and down 50 times at an angle 45, right standing. Inoculated almonds then were placed onto a surface of 2 sheets of absorbent paper (9.125x10.25 inch; Tork, Hand Towel Single fold White) extended inside a sterile plastic tub loosely covered with aluminum foil.

Both sets of inoculated almonds were then allowed subjected to the accelerated drying procedure (Cuervo 2011) or the conventional drying of Danyluk et al. (2005) as described above.

From these two sets, samples of almonds (20g each) were separated after 12 and 24 h, and subjected to Aw measurement in an AquaLab Series 3, Decagon Devices, Inc. water activity meter. Another 20-g sample from each set was also collected for microbiological analysis. The remaining almonds were subjected to various treatments. This procedure was repeated three times in different days, with three replicates each day.

For experiments studying the effect of electron beam irradiation on *Salmonella*, an even dose distribution should be ensured. To achieve small dose uniformity, ratio (DUR), almonds were spot-inoculated by placing 0.1 ml of *Salmonella* Typhimurium LT2 on a single side of each almond. *Salmonella* Typhimurium LT2 was used for the irradiation experiments because the irradiation facility did not allow using biosafety level 2 infectious hazards such as *Salmonella* PT30. *Salmonella* Typhimurium LT2 is a non-virulent strain and is categorized as biosafety level 1. After inoculations, the almonds were dried following the same procedures described above for accelerated drying and conventional drying.

To be able to compare the effect of accelerated drying on the reduction of *Salmonella* on almonds by electron beam irradiation with the thermal treatments (blanching and roasting), another experiment was conducted where almonds were inoculated by dipping as described for the thermal treatments, and subjected to accelerated drying or the drying procedure described by Danyluk et al. (2005).

Sample preparation

After the drying steps described above, inoculated almonds with accelerated drying and conventional drying were subjected to pasteurization treatments consisting of blanching in hot water, oil roasting, and electron beam irradiation. For irradiation treatment the samples were divided into two sets: the first set was vacuum- packaged, double – bagged and was heat sealed and the second set was non- vacuum- packaged and also were heat sealed.

Blanching

In this procedure, a circulating water bath (Polyscience, Niles, IL.) with a 30 x 25 x 15 cm reservoir was filled with distilled water and heated to 88 °C. And the temperature was monitored with a thermocouple and temperature reader (Traceable® Calibration Control Company; Friendswood, TX). A temperature-control almond was prepared by wrapping a thermocouple around the surface of an almond by making a small slit in one side of a single almond and fixing with tape in order to adjust thermocouple with the surface of almond. This device was used to monitor the temperature on the surface of the almonds throughout the heat treatment. Another thermocouple was attached to the water bath to monitor the temperature of water. Samples of 10 g were prepared from inoculated almonds with accelerated drying and the same numbers of samples were prepared from inoculated almonds with conventional drying , one sample at a time was placed into a 12 x 9.5 x 9.5 cm basket and along with the temperature – control the sample was submerge into the water bath. When the temperature reached 88 ± 2 °C on the temperature – control almond, a 4-channel alarm timer (Traceable®) was started. After 10, 20, 30, 40, 50 s the basket with the sample were shaken for 3s in order to remove the excess water and then the almonds were transferred to a sterile stomacher bag containing iced PW and the bag was placed into ice slurry for at least 5 min until the temperature reached 8 ± 0.5 °C for all samples. The detail of cooling temperature for both steps is shown in Table 1.

Then the next sample was started in the same way until the all samples were treated. All samples were then collected, and were subjected to microbiological analysis. This procedure was performed in three replications in three different days.

Oil roasting

In this procedure, samples of 10 ± 2 g were prepared from inoculated almonds with accelerated drying and the same numbers of samples were prepared from inoculated almonds with conventional drying and a deep fryer or a circulating oil bath with 30 x 25 x 15 cm was filled with vegetable oil and was heated to 127 ± 2 °C. Then the samples were placed into a 12 x 9.5 x 9.5 basket, one sample at a time and were submerged into the oil bath. When the temperature control almond reached 127 °C, on the temperature – control almond, a 4-channel alarm timer (Traceable®) were started. After 10, 20, 30, 40, 50 s the basket with the sample were shaken for 3s in order to remove the excess oil, and the almonds were directly placed into a stomacher bag containing ice- chilled PW and immersed into an ice for 5 min until the temperature reached 8 ± 0.5 °C for all samples. The detail of cooling temperature for both steps is shown in Table 1.

Then the next sample was started in the same way until the all samples were treated. All samples were then collected, and were subjected to microbiological analysis. This procedure was performed in three replications in three different days.

Electron beam irradiation

Almonds with accelerated and conventional drying were dispensed in 10-g portions in polyethylene bags and separated in two subgroups. For one subgroup, the bag

was vacuum sealed, whereas no vacuum were applied to the bags in other group. All almonds were placed in the bags on a single-layer- configuration.

Although a non-virulent strain was being used in these experiments, all bags containing almonds were then double- bagged and heat sealed in a special biohazard container following an established protocol by the Agricultural Engineering Laboratory, to prevent pathogen contamination while carrying the samples to the irradiation facility. Both vacuum -packaged and air- packaged almonds were randomly assigned to treatments. Irradiation treatments were carried out using a 1.35 Van de Graaf accelerator (High Voltage Engineering Corporation, Burlington, MA) of the Biological and Agricultural Engineering department, and located in the Hobgood Building at TAMU. Both vacuum packaging almonds and non-vacuum packaging almonds were placed inside a separate cardboard boxes and run through a single e-beam with linear accelerator of 10 MeV. The target doses were 0.25, 0.50, 0.75, and 1.0 kGy and the absorbed dose was verified by placing radiochromic film dosimeters (FWT-60 Series, Far West Technologies, Goleta, CA) inside some of the sample pouches. These dosimeters were read using an electron paramagnetic resonance (EPR) spectroscopy (Bruker EMS 104 EPR analyzer, Bruker Instruments, Germany).

All assays were carried out with three replications on three different days. After treatments, the samples then were placed in an ice-chest with ice-packages and were transported to the Food Microbiology Laboratory for microbiological analysis.

Table 1: The average of cooling temperature for both blanching and oil roasting (n=3)

	Blanching	Oil roasting	
Time (s)	Cooling	Cooling	Cooling tmp^b(C°)
	tmp^a(C°)	tmp^a(C°)	After 5 min
10	16	21	8.2
20	17	24	7.8
30	17	23	8.3
40	19	25	7.9
50	20	26	8.2

n= A number of replications

a Cooling temperature immediately after treatment and sample transferring to polyethylene bag with chilled PW

b Cooling temperature after 5 minutes in ice slurry

Microbiological analysis

After inoculation, 20 ± 2 g of wet almonds was taken to determine the actual concentration of the inoculated bacterium. All samples subjected to blanching, oil roasting, and irradiation treatments were collected by placing the samples in ice-chest for at least 5 minutes, and then were placed into stomach bag and were blended by Stomacher Lab Blender 400 (Model BA6021, A. J. Seward, London, UK) for 1 min. Serial 10-fold dilution of each sample was prepared in sterile 0.1 % peptone water and was surface spread onto TSA + Rif. Plates were incubated at 35 °C for 24 h, and the colonies were enumerated using a Quebec colony counter.

Statistical analysis

All microbial counts (CFU/g) were converted into log values prior to conducting statistical analysis. To obtain reduction results from each replication or D value, the log CFU/g was plotted as a function of time or dose. For the differences between different treatments (irradiation, blanching and oil roasting), an analysis of variance (ANOVA) was used. Comparisons of thermal treatments blanching, oil roasting and irradiation were analyzed by applying the slope comparison procedure on the regression lines, using the general linear model. For comparison between means, the general linear model was used and an alpha of 0.5 was used to determine significance. All statistical analyses were performed using JMP statistical software (version10) (SAS Institute, Cary, NC, USA).

CHAPTER III

RESULTS AND DISCUSSION

Results and discussion

Preliminary study

The growth of *Salmonella* on TSA, TSA+Rif and TSA+NaCl was compared for almonds that were subjected to accelerated drying (Cuervo 2011) or conventional drying (Danyluk et al. 2005). No significant differences in the growth characteristics of *Salmonella* were found between different types of media, *Salmonella* reached the same maximum population density in all three types of media as shown in Tables 2 and Table3.

To compare between accelerated drying and conventional drying and the effect of A_w on reduction of *Salmonella*, the A_w of inoculated almonds was measured after 3, 6, 9, 12, and 24 h of drying. The results showed that A_w was higher in case of conventional drying. Greater reductions of *Salmonella* were observed in case of accelerated drying than conventional drying.

After two outbreaks of salmonellosis in USA and Canada implicated with consumption of raw almonds, the scientific community, together with the almond-industry started conducting research to develop effective methods to eliminate *Salmonella* from the almonds (Chang et al. 2010, Bari et al. 2009, Bari et al. 2010, Pao et al. 2006, Jeong et al. 2009, Danyluk et al. 2005, Willford et al. 2008, Lee et al. 2006, Deng et al. 2007, Brandl et al. 2008, Uesugi et al. 2006, Harris L.J. et al. 2011,

Goodridge et al. 2006, Yang et al. 2010). Because these studies often have ignored the increase in water resulting from the inoculation of almonds. In the present study, the effect of accelerated drying was evaluated by comparing the almonds that were subjected to accelerated drying (Cuervo 2011) or conventional drying (Danyluk et al. 2005) to determine the affect of accelerated drying on *Salmonella* reduction on current almonds pasteurization methods blanching, roasting, and electron beam irradiation.

Water activity, moisture and accelerated drying

The data on Table 4 shows the effect of accelerated drying to bring the Aw of almonds from 0.81-0.82 immediately after inoculation, to 0.58 after 12 h in the incubator at 35°C. This value is slightly lower than the baseline of 0.66 for the raw almonds. In contrast, the Aw of the almonds that were dried following Danyluk et al. (2005) procedure was 0.80, almost identical to the value after inoculation. In fact, only little, if any, reduction in Aw was observed by applying Danyluk et al.'s procedure after 12 and 24 h post inoculation. In order to compare the measurements, the almonds that were subjected to accelerate drying were placed in a desiccator after the 12 h drying period. This step was indented to maintain the Aw without change until completing 24 h to enable comparison between the two drying methods.

After 24 h, the Aw of almonds with accelerated drying ranged from 0.59 to 0.55 while in case of conventional drying (no Aw restoration) the Aw ranged from 0.75 to 0.8. The survival, of *Salmonella* in the almonds using drying by the accelerated drying or conventional procedures is shown in Figure 1. The trend does not show differences in behavior of this organism as affected by the drying method. In addition, the statistical

analysis showed no differences in the survival as are shown in Table 5. More studies will be needed to assess the effect of moisture reduction on the survival of *Salmonella* on almonds.

The moisture content for both accelerated and conventional drying are shown in Table 6, the average of moisture content for almonds with accelerated drying was 7.4% after 12 h in the incubator while in case of conventional drying the average of moisture content was 10.1%. And after 24 h of drying, the average of moisture content for almonds with accelerated drying was still 7.4% while the average of moisture content was 8.9% in case of almonds with conventional drying.

Table2: *Salmonella* reduction over the time in different media with accelerated drying

Time	TSA	TSA+rif	TSA+NaCl
0	6.8 ^a	6.8 ^a	6.6 ^a
3	4.8 ^a	4.8 ^a	4.7 ^a
6	4.5 ^a	4.4 ^a	4.3 ^a
9	4.9 ^a	4.9 ^a	4.5 ^a
12	4.4 ^a	4.4 ^a	4.3 ^a
3 days	3.7 ^a	3.7 ^a	3.8 ^a

TSA : Tryptic soy agar

TSA +rif : tryptic soy agar with rifampicin

TSA+ NaCl : tryptic soy agar with sodium chloride

Means in the same row with the same letter (a, b, or c) are not significantly different ($P > 0.05$)

Table3: *Salmonella* reduction over the time in different media with conventional drying

Time	TSA	TSA+rif	TSA+NaCl
0	6.7 ^a	6.6 ^a	6.6 ^a
3	3.9 ^a	3.3 ^b	3.8 ^a
6	4.4 ^a	4.5 ^a	4.2 ^a
9	4.8 ^a	4.7 ^a	4.3 ^a
12	5 ^b	5 ^b	4.6 ^a
3 days	4.4 ^a	3.8 ^a	4 ^a

TSA : Tryptic soy agar

TSA +rif : tryptic soy agar with rifampicin

TSA+ NaCl : tryptic soy agar with sodium chloride

Means in the same row with the same letter (a, b, or c) are not significantly different ($P > 0.05$)

Table 4: Water activity measurement with accelerated and conventional drying

Time	Aw with accelerated	Aw with conventional
Baseline	0.66 ^a	0.66 ^a
Inoculated almonds	0.813 ^b	0.816 ^b
After 12h	0.576 ^c	0.815 ^d
After 24h	0.56 ^c	0.80 ^d
After 4days	0.561 ^c	0.543 ^c

Aw is water activity

Aw with conventional drying inoculated almonds were placed at 23±2°C

Aw with accelerated drying inoculated almonds were placed inside incubator for 12 h at 35 °C

Means in the same row with the same letter (a, b, or c) are not significantly different (P > 0.05)

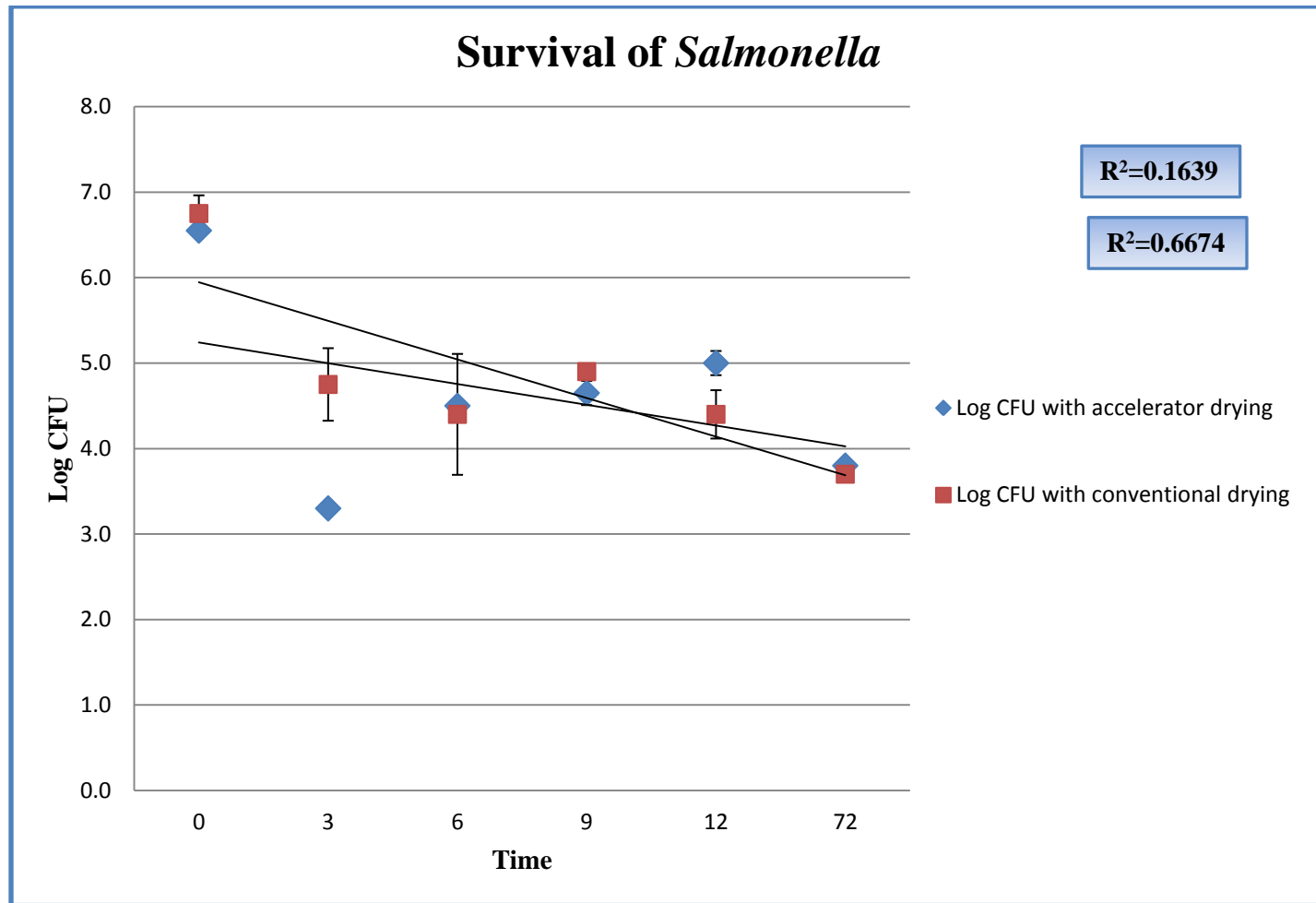


Figure 1: The survival of *Salmonella* after drying procedures (with accelerated and conventional drying)

Table 5: The survival of *Salmonella* after accelerated and conventional drying (n=4)

Time	Log CFU with accelerated drying*	Log CFU with conventional drying*
0 h	6.6a	6.8a
3 h	3.3a	4.8b
6 h	4.5a	4.4a
9 h	4.7a	4.9a
12h	5.0a	4.4a
72 h	3.8a	3.7a

n= A number of replications

* The survival of *Salmonella* after each time with both accelerated and conventional drying

Means in the same row with the same letter (a, b, or c) are not significantly different ($P > 0.05$)

Table 6: The moisture content for almonds with accelerated and conventional drying

Almonds	Moisture % with accelerated drying	Moisture with conventional drying
The base line	7.6 ^a	7.6 ^a
Inoculated almonds	14.3 ^b	14.3 ^b
After 12 h	7.4 ^c	10.1 ^d
After 24 h	7.4 ^c	8.9 ^e

Means in the same row with the same letter (a, b, c or d) are not significantly different ($P > 0.05$)

Means in the same column with the same letter (a, b, c or d) are not significantly different ($P > 0.05$)

Thermal reduction of Salmonella

After *Salmonella* outbreaks that were linked to the consumption of raw almonds, an action plan have been implemented by Federal Law through California almond board, has mandated a pasteurization of all raw almonds in order to achieve at least 4- log reduction of *Salmonella* (Federal Register 2007). Therefore, the industry and scientific community have investigated the efficacy of the most effective methods to control *Salmonella* in almonds. Currently, the FDA has approved the blanching and oil roasting technique as pasteurization intervention of almond.

Blanching

As previously mentioned blanching is one of the most common pasteurization methods of almonds, and it is heat treatment in hot water to up 88°C. Therefore, inoculated almonds were subjected to blanching treatment to eliminate *Salmonella*. After 24 hours from inoculation of almonds, almond samples for both batches; almonds with accelerated drying and almonds conventional drying were subjected to heat to up to 88°C. Blanching thermal inactivation curves at 88°C were structured for each of batches and fitted to a linear regression equation. From the curves shown in Figure 2, D- values were calculated as a negative inverse of slope and compared between almonds with accelerated drying and almonds conventional drying. The mean and SD of the D-value for blanching almonds with accelerated drying was 10.7 ± 0.3 s, and for almonds with conventional drying was 12.8 ± 0.2 s. The data from blanching with accelerated and conventional drying) Figure 2 showed less scattering, which resulted in acceptable values of $R^2 = 0.91, 0.94$, respectively. These data should be used to calculate the D

values. This experiment showed no significant differences between blanching almonds with accelerated drying and blanching almonds with conventional drying. However, a greater reduction of *Salmonella* was obtained by blanching with accelerated drying Figure 4. Harris et al. (2011) evaluated the efficacy of blanching as an intervention to control *Salmonella* in almonds, and suggested that in order to achieve 5-log reduction of *Salmonella* in almonds; two min were needed to perform pasteurization. Based on this research, the FDA agreed to blanch for 2 min at 88 °C as the commercial industry standard pasteurization of almonds (ABC 2007). However, according to results obtained from the present study, the minimum time required for blanching raw almonds may be less than 2 min. Sixty- four second was the time required to achieve a 5-log reduction, because more reduction of *Salmonella* was observed in case of accelerated drying which is closest to the actual condition of the almonds and *Salmonella* was below the detectable limit in case of almonds with accelerated drying. Thus, the sensory and nutritional quality of almonds might be affected when almonds are exposed to blanching for 2 min. According to the results of this study, adequate almond pasteurization by blanching should be achieved in less than 1.2 min, and 2 min may be more treatment time than the necessary to comply with pasteurization requirements in almonds.

Oil roasting

Inoculated almonds both with accelerated drying and conventional drying were subjected to heat treatment in hot oil at 127°C. The thermal inactivation curves for oil roasted almonds are shown in Figure 3. The mean and SD for almonds with accelerated drying was 10.5 ± 0.1 s and with conventional drying was 10.2 ± 0.2 s. The data from oil

roasting with accelerated and conventional drying) Figure 3 showed more scattering and dispread far from the line. However, these data resulted in acceptable values of $R^2 = 0.86, 0.87$, respectively. These data should be used to calculate the D values. Therefore, according to this study the minimum time required to achieve 5-log reduction was 55 s at 127°C. Based on the data from this study no significant differences between oil roasting with accelerated drying and oil roasting with conventional drying were found ($p>0.05$). Du et al. (2010) examined the effectiveness of oil roasting as thermal treatment for inoculated almonds with *Salmonella* Enteritidis PT 30, suggesting that 1.5 min at 127 °C is required to achieve 5-log reduction.

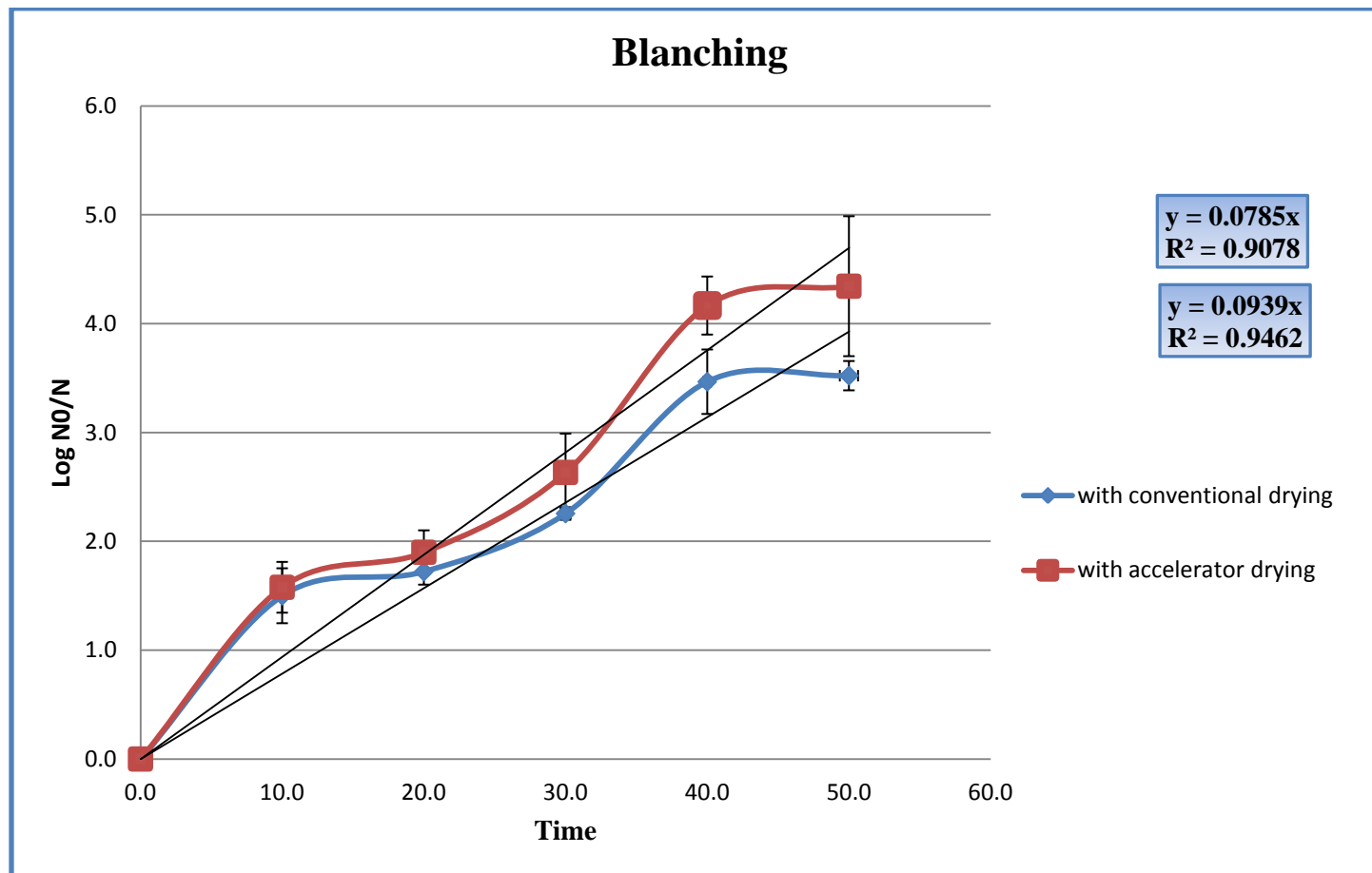


Figure 2: Thermal inactivation curves for blanching with accelerated and conventional drying

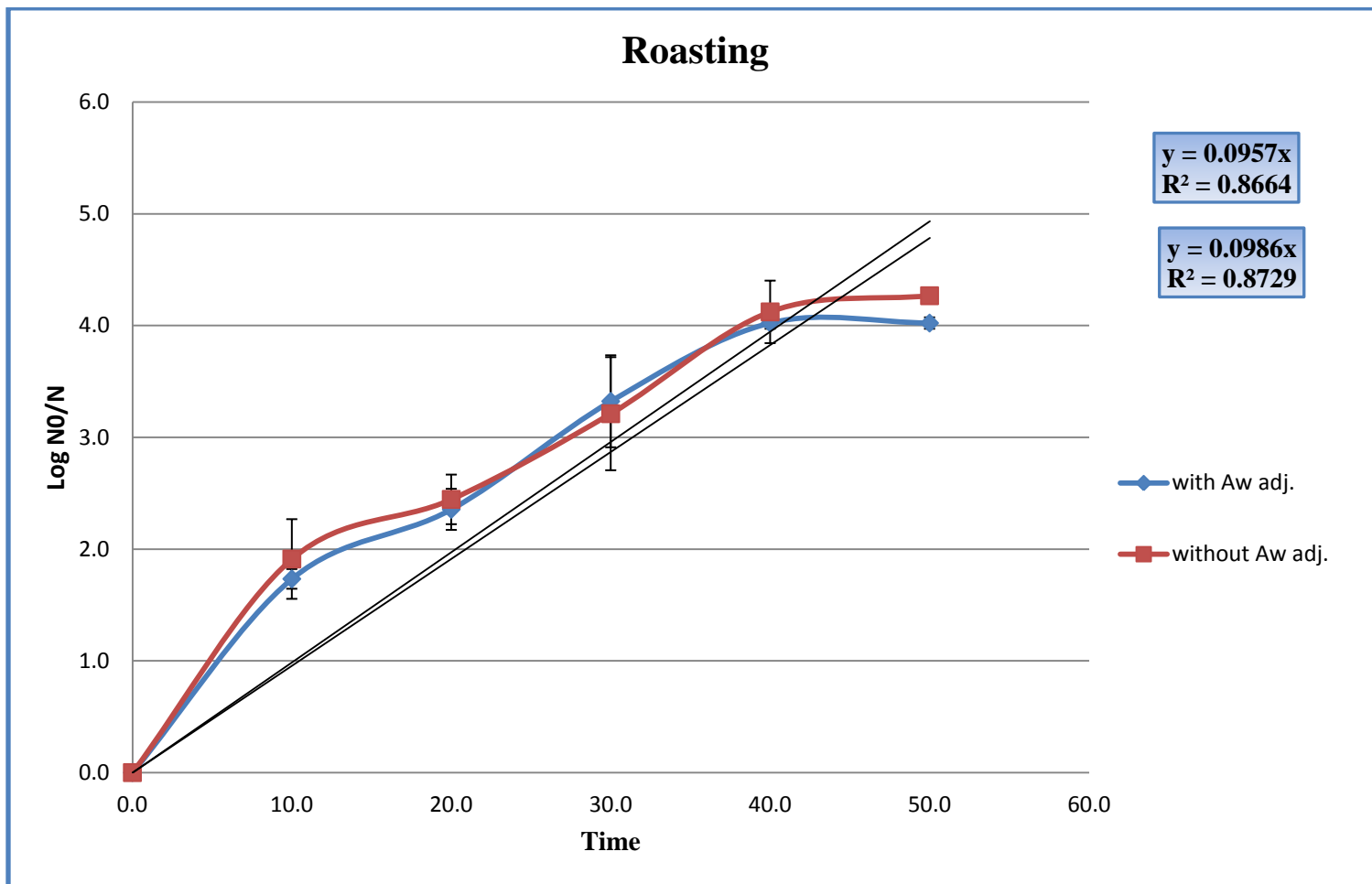


Figure 3: Thermal inactivation curves for roasting with accelerated and conventional drying

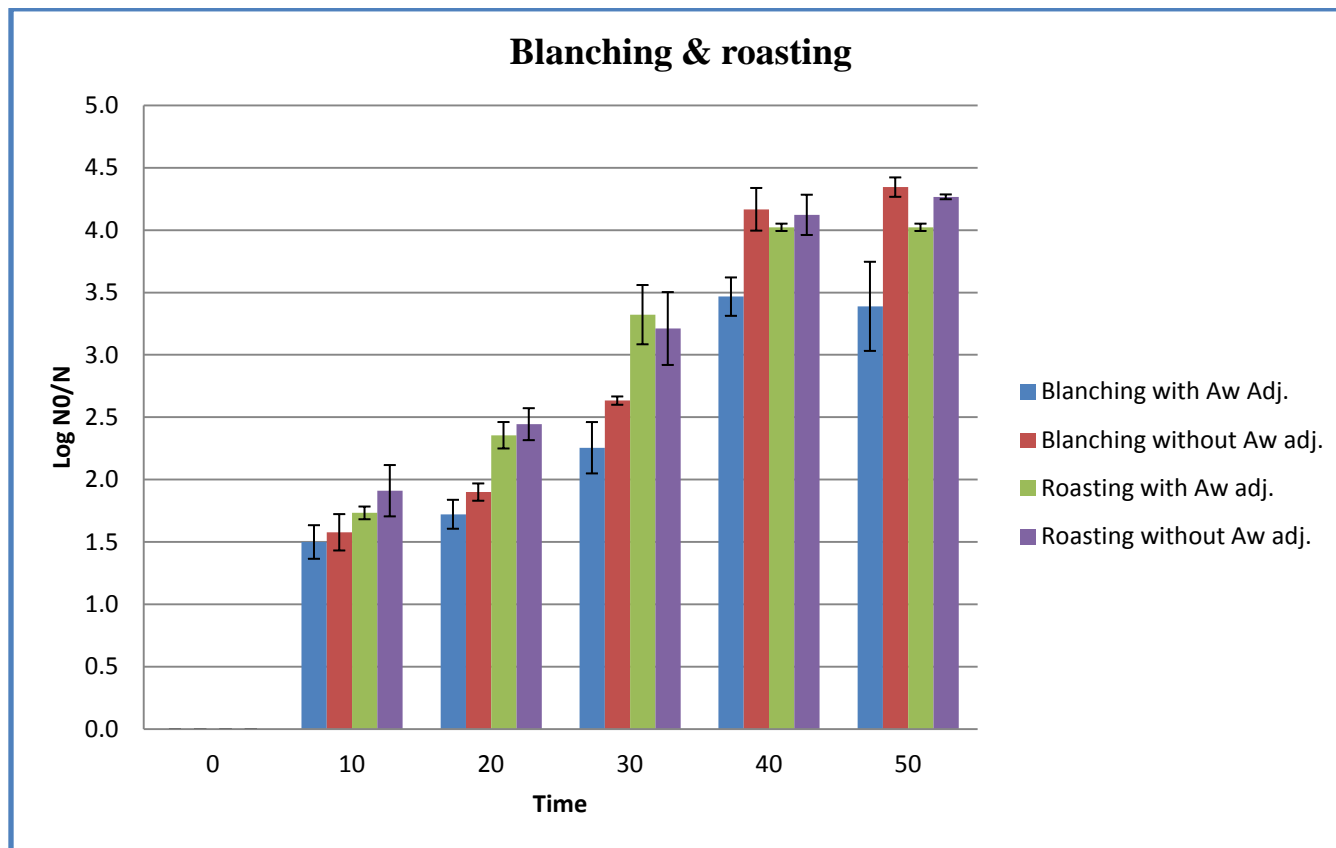


Figure 4: The effect of accelerated drying on reduction of *Salmonella* to treatments

Decimal reduction dose of *Salmonella* on inoculated almonds using electron beam irradiation

Irradiation

In this study, inoculated almonds surface with *Salmonella* for both atmospheric conditions vacuum and air and both batches of almond with accelerated and conventional drying were subjected to electron beam irradiation and compare to each other using a single beam 1.35 MeV Van de Graaf accelerator. The mean and SD of the doses applied were 1.00 ± 0.001 , 0.752 ± 0.002 , 0.499 ± 0.001 , 0.250 ± 0.001 . The dosimetry or the calculated doses for the spot inoculated samples are shown in Table 7. To determine the D10-values of the *Salmonella*, irradiation inactivation curves were constructed for each situation including vacuum packaging and air packaging, with accelerated and conventional drying (Figures 5 and 6), and linear regression analysis was applied to all of data. From the inactivation curves, D10-values were calculated and compared between vacuum with accelerated and conventional drying, and between air with accelerated and conventional drying. As can see on (Figure 7) 0.5 kGy did not show difference between accelerated and conventional drying in both cases (air and vacuum packaged); but higher doses in case of air did show differences between accelerated and conventional drying. This may be due to the fact that at higher dose, the presence of more free water in case of conventional drying retards the dose penetration. Then comparison between the mean D10-values of vacuum and air were performed (Figure7).The mean D10-value and SD for vacuum with accelerated and conventional drying were 0.35 ± 0.02 kGy, 0.38 ± 0.04 kGy respectively. And mean of D10-values and

SD for air with accelerated and conventional drying were 0.26 ± 0.04 kGy, 0.29 ± 0.03 kGy respectively. The data from vacuum packaged (Figure 5) showed very scattered and dispread, which resulted in unacceptable values of $R^2 = 0.76$ and 0.75 , respectively. These data are not useful for calculating D values. However, the data from air packaged (Figure 6) showed less scattering, which resulted in acceptable values of $R^2 = 0.93$ and 0.88 , respectively. These data can be used to calculate the D values. No significant differences were found between irradiation in vacuum packaged with accelerated and conventional drying, and air packaged with accelerated and conventional drying $p > 0.05$. Based on the results presented above, the average of the D_{10} values for *Salmonella* LT2 inoculated on the surface of almonds and irradiated under vacuum is 0.36 ± 0.03 kGy. And the D_{10} values for *Salmonella* LT2 inoculated on the surface of almonds and irradiated under atmospheric conditions is 0.28 ± 0.04 kGy. The A_w values were also not different for almonds with accelerated and conventional drying. This may be explained by the fact that in this case, the almonds were inoculated by placing a small volume on a small spot of the almond surface, and this may induce a faster drying of the almonds even if not placed in incubator. From the inactivation curve significant differences between vacuum and air were found ($p < 0.05$). This confirmed that the presence of oxygen affects the sensitivity of *Salmonella* to electron beam irradiation. This study indicated that in order to achieve 4-log reduction of *Salmonella* on the surface of almond using irradiation, only 1.05 kGy is required in the presence of oxygen and 1.56 kGy is need in absence of oxygen. The results of this study is contrary to the results that were shown by Prakash et al. (2010), which demonstrated that D-value for

inoculated almonds with *Salmonella* Enteritidis PT 30 is 1.25 kGy . Also indicated that 5.0 kGy is required in order to perform 4-log reduction which may induce significant sensory and nutritional changes in almonds. Additionally, this large dose affects the feasibility of irradiation as non-thermal treatment to inactivate *Salmonella* in almonds because outcomes lead to the consumer's dissatisfaction and unacceptable almonds.

When the inoculation was conducted by dipping, both sides of inoculated almonds were exposed to only one single dose, and the effect of accelerated drying was recorded as log reduction (Figure 8). The data from dip inoculation showed very less variation for accelerated and conventional drying, which resulted in acceptable data that can be used for calculating log reduction; it is acknowledged that the dose distribution over the round almond surface most likely was not as uniform as for spot inoculation. However, this method was also used to enable comparison between the various treatments applied. The DUR obtained from the simulation was 1.90. According to Moreno and others (2007), the DUR was within the acceptable industry standards. This study indicated that a DUR of 2.4 is an acceptable range in commercial applications.

Figure 8 showed no differences between irradiated under vacuum with accelerated vs. conventional drying, and between air with accelerated vs. conventional drying. Since Figure 7 clearly shows that there may be differences, future research using dip inoculation should include more doses to determine a possible combined effect of the dose and accelerated drying on the reduction of *Salmonella*.

The ANOVA showed no significant difference between counts with accelerated and conventional drying. In summary, irradiation may be a feasible non-thermal

intervention to eliminate *Salmonella* from almonds, and a great reduction can be obtained if irradiation is applied in the presence of oxygen. This study indicated that irradiation may be effective with less dose compared to previous studies.

Table 7: The calculated dose for spot inoculated samples

Sample	Dose [kGy]	
	Target	Measured
Vac- with accelerated drying	1.00	0.99±0.02
Vac- with conventional drying	1.00	0.99±0.02
Air with accelerator drying	1.00	0.99±0.02
Air with conventional drying	1.00	1.00±0.02
Vac-with accelerated drying	0.75	0.75±0.02
Vac- with conventional drying	0.75	0.75±0.02
Air with accelerated drying	0.75	0.75±0.02
Air with conventional drying	0.75	0.75±0.02
Vac- with accelerated drying	0.50	0.50±0.02
Vac- with conventional drying	0.50	0.50±0.02
Air with accelerated drying	0.50	0.49±0.02
Air with conventional drying	0.50	0.50±0.02
Vac- with accelerated drying	0.25	0.25±0.02
Vac- with conventional drying	0.25	0.24±0.02
Air with accelerated drying	0.25	0.24±0.02
Air with conventional drying	0.25	0.25±0.02

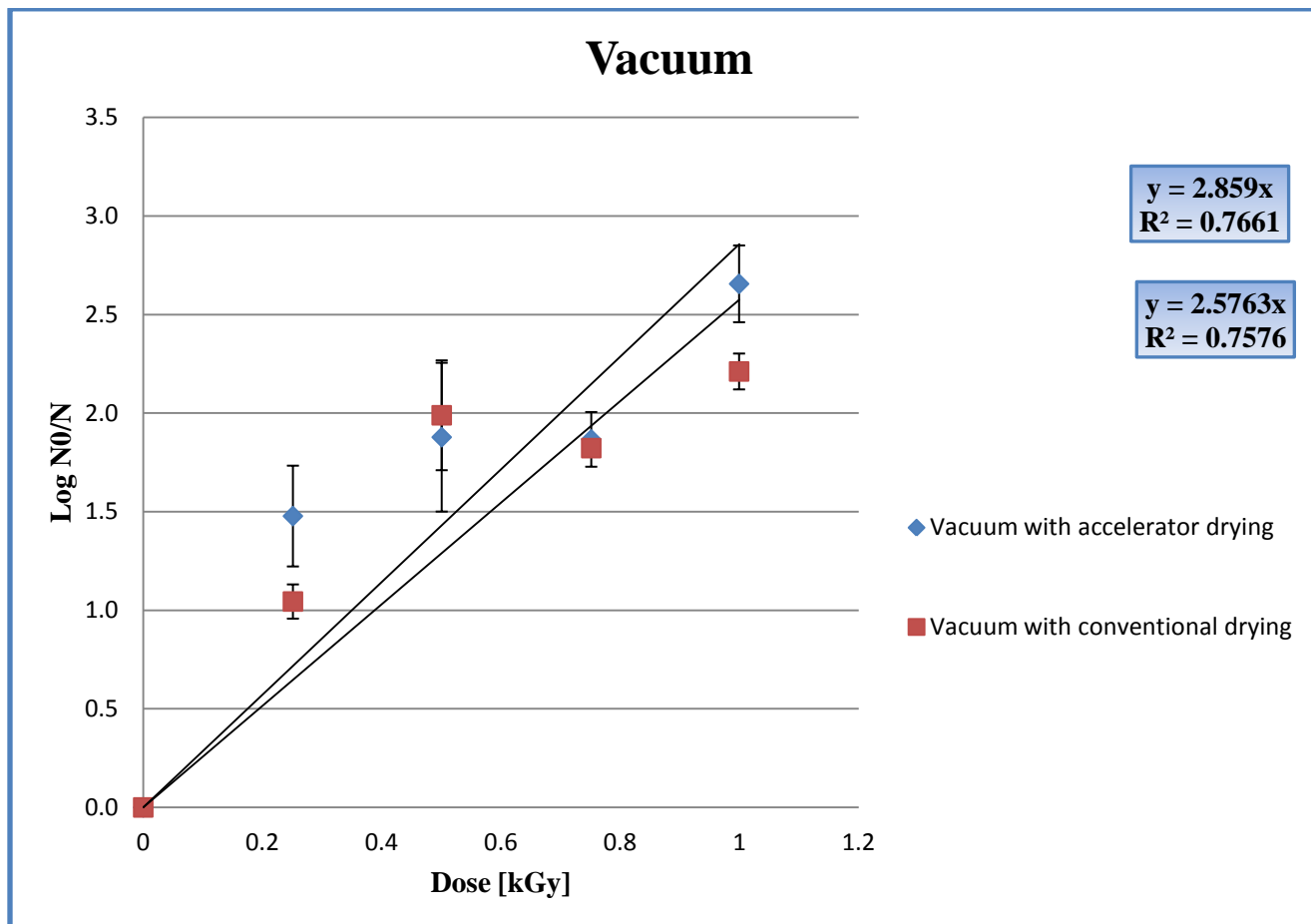


Figure 5: Comparison between vacuum with accelerated and conventional drying

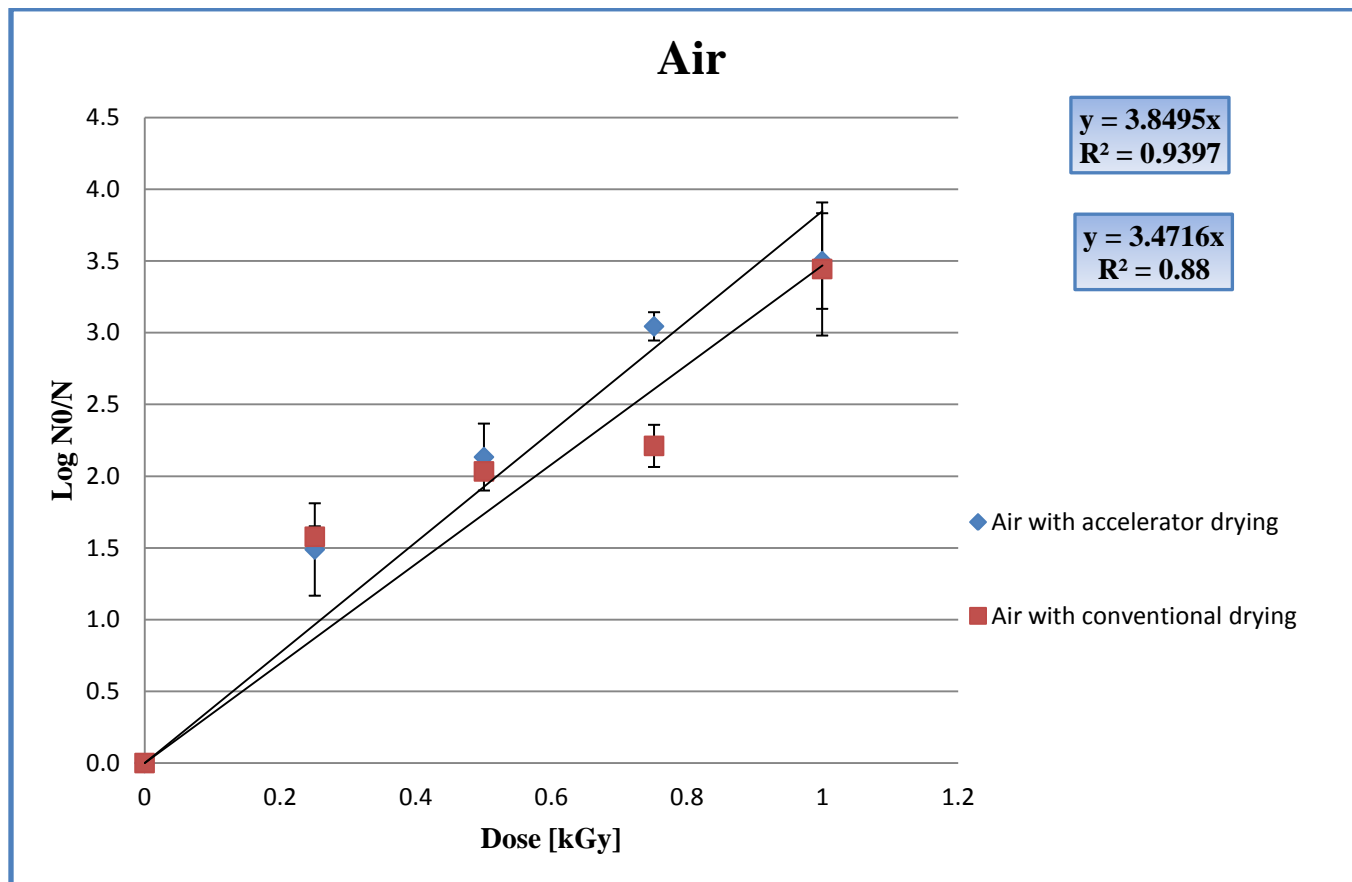


Figure 6: Comparison between air with accelerated and conventional drying

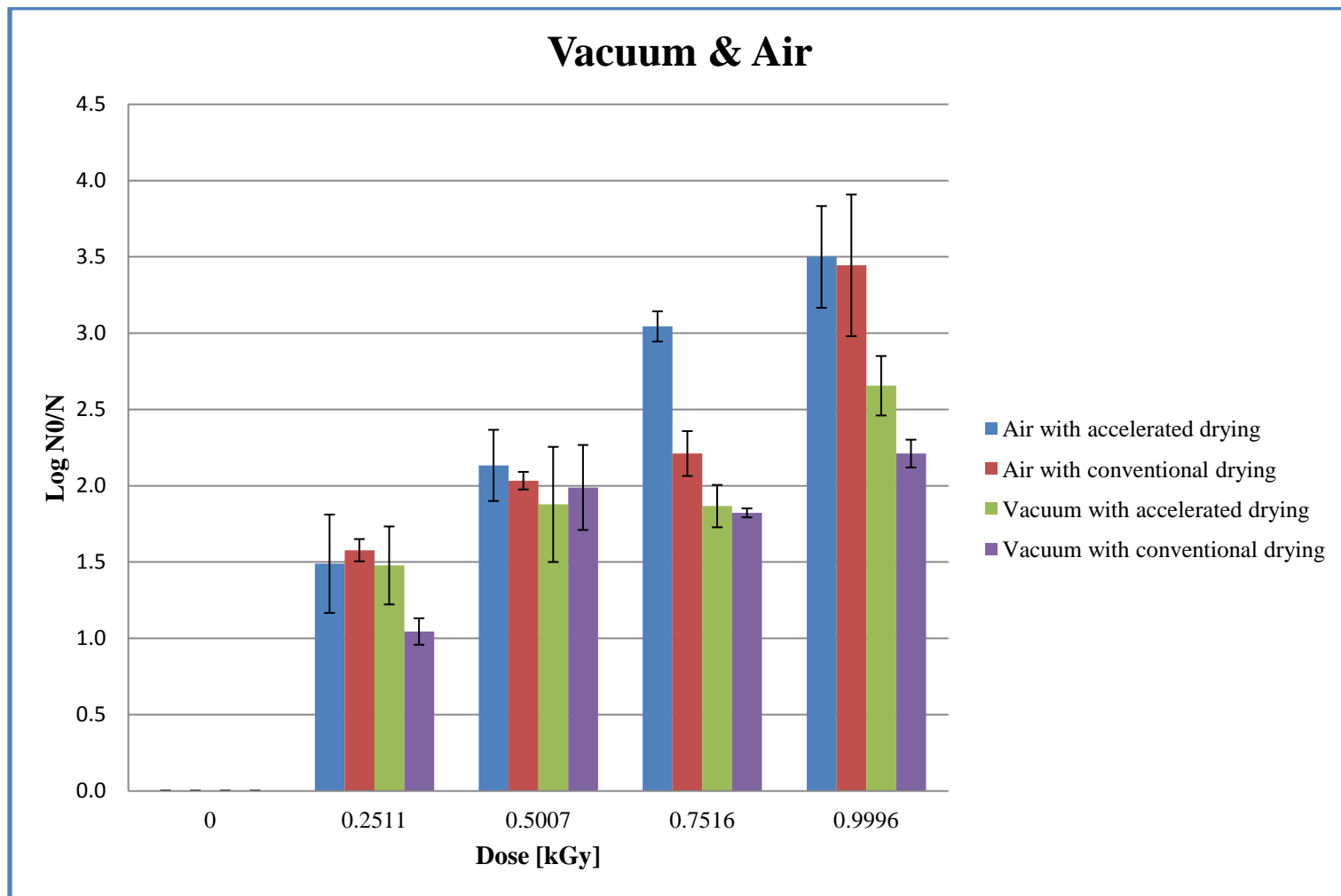


Figure 7: The effect of vacuum and air atmosphere on the sensitivity of *Salmonella* to electron beam irradiation

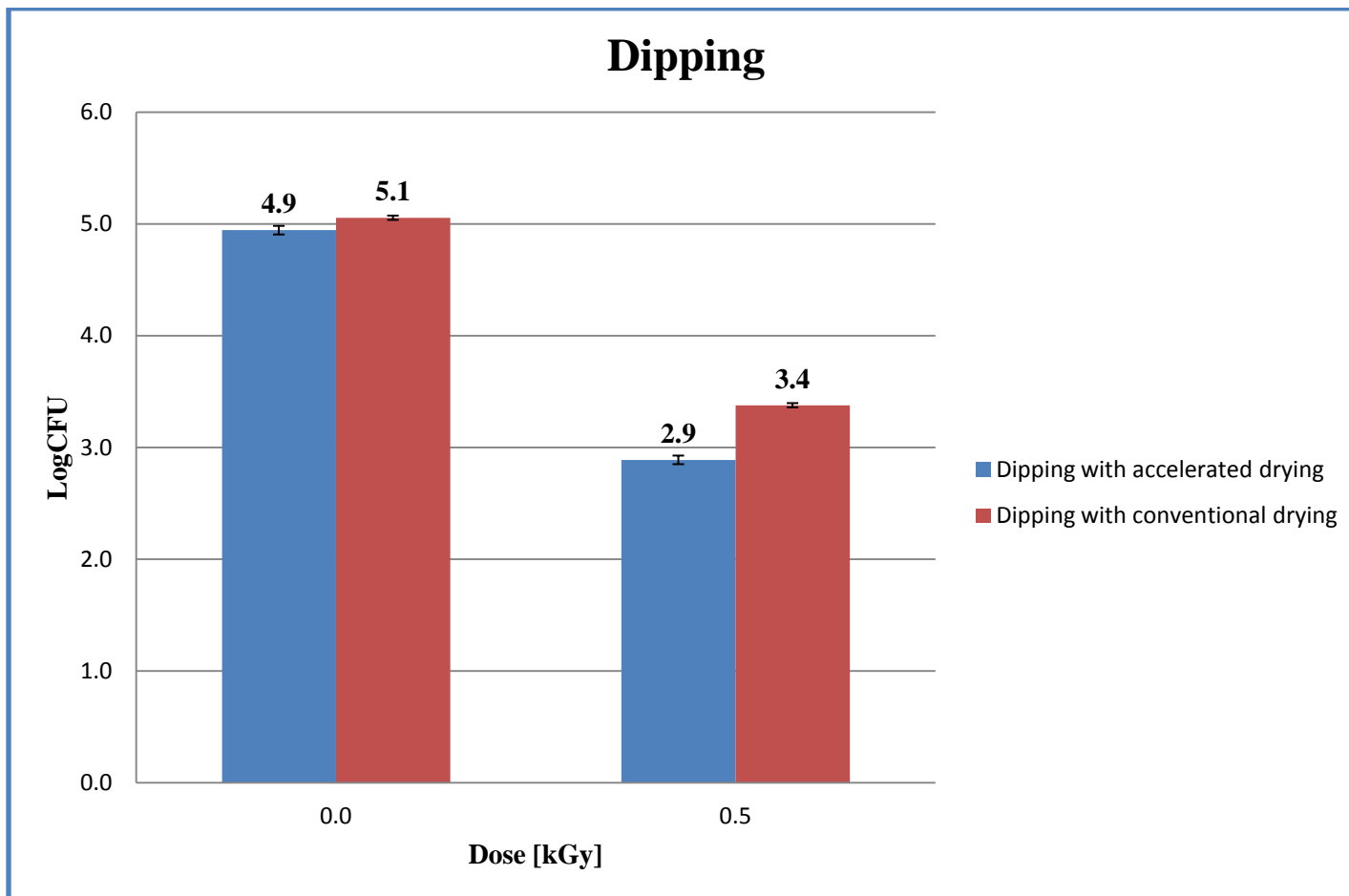


Figure8: The effect of accelerated drying on reduction of *Salmonella* to electron beam irradiation

CHAPTER IV

CONCLUSIONS

In this study, the effect of accelerated drying on sensitivity of *Salmonella* to current methods for thermal pasteurization of almonds, and to electron beam irradiation was evaluated. No significant differences were found between blanching or roasting for almonds with accelerated and conventional drying. In the case of irradiation, when almonds were spot inoculated, significant differences were found between surfaces measured under vacuum vs. air. Based on the results from this study, the average D-value for *Salmonella* Enteritidis PT30 inoculated on the surface of almonds and then dried by accelerated and conventional drying 11.8 ± 0.3 s when using blanching at 88 °C and 10.4 ± 0.2 s when treated by oil roasting at 127 °C. No differences were observed in the reductions as affected by accelerated or conventional drying ($p > 0.05$).

. In the present work, the effect of vacuum and air packaging were also investigated for their effect on the sensitivity of *Salmonella* to e- beam irradiation. The data showed that the presence of oxygen was essential to obtain a greater reduction of *Salmonella* after electron beam irradiation. The reductions obtained for vacuum packaged almonds were significantly smaller than the reductions obtained for almonds that were packaged without vacuum, ($p < 0.05$).

In summary, the average of the D_{10} values for *Salmonella* LT2 inoculated on the surface of almonds and irradiated under vacuum was 0.36 ± 0.03 kGy. Whereas the D_{10}

values for *Salmonella* LT2 inoculated on the surface of almonds and irradiated under atmospheric conditions was 0.28 ± 0.04 kGy.

This study indicated that current almond pasteurization blanching, oil roasting and electron beam irradiation may be used to achieve 4 to 5- log reductions of *Salmonella* in raw almonds with less time or, dose in comparison with the industry standard pasteurization procedures. The industry standard pasteurization for blanching and oil roasting are 2 min and 1.5 min respectively. For irradiation 3.6 kGy or higher was recommended for almonds. These recommendations were developed on the basis of the results from previous studies. This study found that the time required for blanching and oil roasting is less than one min to achieve 5-log reduction of *Salmonella* on the surface of almonds. Only 1.8 kGy is needed for irradiated the surface of almonds under vacuum, and 1.4 kGy is needed when irradiated under air condition. More research is needed to confirm these observations and to determine the benefits, if any, of reducing the time or temperature of pasteurization treatments. If these conclusions are true, then significant savings in time and energy might be achieved by adjusting the procedures accordingly. Studies focusing on the conditions under dry environment are needed. Furthermore, more research on the effect of presence of oxygen on the lipid oxidation at lower irradiation dose and the effect of that on nutrition and sensory quality of almonds is needed. Scientific evaluation of environmental conditions such as (temperature, humidity) that related to outbreaks that linked to almond consumption or presence of *Salmonella* is needed to compare the differences in environmental changes in recent years.

REFERENCES

- [ABC] Almond Board of California. 2007. Almond action plan, CA. [Accessed 2013 May 15]. Available from:
<http://www.almondboard.com/Handlers/Documents/Pasteurization-Treatments.pdf>
- [ABC] Almond Board of California. 2009. California Almonds facts, CA. [Accessed 2013 May 15]. Available from:
<http://ucce.ucdavis.edu/files/datastore/608-3.pdf>
- [ABC] Almond Board of California. 2012a. Almond Board: Pasteurization of Almonds – almond pasteurization, CA. [Accessed 2013 May 15]. Available from:
<http://www.almondboard.com/Handlers/FoodQualitySafety/Pasteurization/Pages/Default.aspx>
- [ABC] Almond Board of California. 2012b. Almond Almanac: Modesto, CA. [Accessed 2013 May 15]. Available from:
<http://www.almondboard.com/Handlers/FoodQualitySafety/Pages/default.aspx>
- [ABC] Almond Board of California. 2012c. Almond Almanac: Modesto, CA. [Accessed 2013 May 15]. Available from:
<http://www.almondboard.com/Growers/lawsandregulations/FoodSafety/Pages/Default.aspx>
- [ABC] Almond Board of California. 2012d. Almond Almanac, This annual publication summarizes the production statistics of the California almond industry. [Accessed 2013 May 15]. Available from:

http://www.almondboard.com/AboutTheAlmondBoard/Documents/2012%20Almond%20Almanac_FINAL.pdf

Abee, T., and J. A . Wouters. 1999. Microbial stress response in minimal processing. Intl. J Food Microb. 50:65-91.

[AMS] Agricultural Marketing Service. 2010. Marketing Orders. USDA [Accessed 2013 May 15]. Available from:
<http://www.ams.usda.gov/AMSV1.0/ams.fetchTemplateData.do?template=TemplateN&page=FVMarketingOrderIndexAlmonds>.

Aavitsland P., O. Alvseike, P.J. Guérin, and T.L. Stavnes. 2001. International outbreak of *Salmonella* Typhimurium DT104 – update from Norway. Euro Surveill. 5:1701. .
[Accessed 2013 May 15]. Available from:
<http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1701>

Afshari Roya, Hedayat Hosseini. 2012. Atmospheric pressure plasma technology: a new tool for food preservation. IPCBEE 33: IACSIT Press, Singapore

Barbosa-Canovas, G. V., Pothakamury, U. R., Palou, E., & Swanson, B. G. 1998.
Nonthermal preservation of foods. New York:Marcel Dekker.

Bari ML, Nei D, Sotome I, Nishina I, Isobe S and Kawamoto S. 2009. Effectiveness of sanitizers, dry heat, hot water, and gas catalytic infrared heat treatments to inactivate *Salmonella* on almonds. Foodborne Patog Dis 8:953-8.

Bari L, Nei D, Sotome I, Nishina IY, Hayakawa F, Isobe S, Kawamoto S. 2010.
Effectiveness of superheated steam and gas catalytic infrared heat treatments to inactivate *Salmonella* on raw almonds. Foodborne Patog Dis 7:845-50.

- Bermúdez-Aguirre D. and Maria G. Corradini.2012. Inactivation kinetics of *Salmonella* spp. under thermal and emerging treatments: A review. Food Res. Int. 45: 700–712.
- Bingol, G., J. Yang, M.T. Brandl, Z. Pan, H. Wang, and T.H. McHugh. 2011. Infrared pasteurization of raw almonds. J Food Eng. 104: 387-393.
- Brandl MT, Pan Z, Huynh S, Zhu Yi, McHugh T. 2008. Reduction of *Salmonella* Enteritidis population sizes on almond kernels with infrared heat. J Food Prot 71:897-902.
- Brockmann S. 2001. International outbreak of *Salmonella* Typhimurium DT104 due to contaminated sesame seed products – update from Germany (Baden-Württemberg). Euro Surveill. 5:1699. [Accessed 2013 May 15]. Available from:
<http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1699>
- Bruhn C., L. Harris, Maria G., Diane M..2010. Nuts: Safe methods for consumers to handle, store, and enjoy. University of California, ANR catalog, Agriculture and Natural Resources. 13: 978-1-60107-685-4.
- Beuchat L. R , Evangelia K., Harry B.s, Roy P B., François B., Séamus F., Han M J., Benno H Ter Kuile.2013 . Low-water activity foods: increased concern as vehicles of foodborne pathogens. J Food Prot 76:150-72
- Boriss H. , Henrich B.. 2012. Almond profile. AgMRC resource center. [Accessed 2013 May 15]. Available from:
http://www.agmrc.org/commodities_products/nuts/almond-profile/
- Cavallaro, E., Date, K., Medus, C., Meyer, S., Miller, B., Kim, C., Nowicki, S.,. 2011.*Salmonella* Typhimurium infections associated with peanut products. N. Engl. J Med., 365: 601–610.

California Department of Public Health. 2004. Environmental investigation of *Salmonella* Enteritidis Phage Type 9c outbreak associated with consumption of raw almonds.

[Accessed 2013 May 20]. Available from:

<http://www.cdph.ca.gov/pubsforms/Documents/fdb%20eru%20Almond%20SE2004.pdf>

Centres for Disease Control and Prevention (CDC). 1993. *Salmonella* serotype Tennessee in powdered milk products and infant formula – Canada and United States, 1993. Morb. Mortal. Wkly Rep, 42: 516-517.

Centres for Disease Control and Prevention (CDC). 1998. Multistate outbreaks of *Salmonella* serotype Agona infections linked to toasted oats cereal – United States, April – May, 1998, Morb. Mortal. Wkly Rep., 47: 462-464.

Centers for Disease Control and Prevention. 2004. Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds—United States and Canada, 2003–2004. Morb. Mortal. Wkly. Rep. [Accessed 2013 May 20]. Available from: <http://www.cdc.gov/mmwr/PDF/wk/mm5322.pdf>.

Centers for Disease Control and Prevention. 2007a. Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter—United States 2006–2007. MMWR 56: 521-524. [Accessed 2013 May 20]. Available from: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5621a1.htm>

Centers for Disease Control and Prevention. 2007b. Salmonellosis--Outbreak investigation February 2007. [Accessed 2013 May 20]. Available from: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_2007/outbreak_notice.htm

Centers for Disease Control and Prevention. 2008. Investigation of outbreak of infections caused by *Salmonella* Agona. [Accessed 2013 May 20]. Available from: www.cdc.gov/print.do?url=http%3A//www.cdc.gov/Salmonella/agona.

Centers for Disease Control and Prevention. 2009a. Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter-containing products—United States, 2008-2009. MMWR 58: 85-90. [Accessed 2013 May 20]. Available from <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5804a4.htm>

Centers for Disease Control and Prevention. 2009b. Investigation update: Outbreak of *Salmonella* Typhimurium infections, 2008-2009. [Accessed 2013 May 20]. Available <http://www.cdc.gov/Salmonella/typhimurium/archive/031009.html>

Centers for Disease Control and Prevention. 2011. Investigation announcement: Multistate outbreak of E. coli O157:H7 infections associated with in-shell hazelnuts. [Accessed 2013 May 20]. Available <http://www.cdc.gov/ecoli/2011/hazelnuts0157/index.html>

Centers for Disease Control and Prevention. 2011b. Multistate outbreak of human *Salmonella* Enteritidis infections linked to Turkish pine nuts. [Accessed 2013 May 20]. Available <http://www.cdc.gov/Salmonella/pinenuts-enteritidis/111711/index.html>

Centers for Disease Control and Prevention. 2012. Multistate outbreak of *Salmonella* Bredeney infections. September 25, [Accessed 2013 May 20]. Available <http://www.cdc.gov/Salmonella/bredeney-09-12/index.html>

Centers for Disease Control and Prevention. 2013. *Salmonella*. [Accessed 2013 May 18]. Available <http://www.cdc.gov/Salmonella/general/>

- Chan, E. S., J. Aramini, B. Ciebin, D. Middleton, R. Ahmed, M. Howes, I. Brophy, I. Mentis, F. Jamieson, F. Rodgers, M. Nazarowec-White, S. C. Pichette, J. Farrar, M. Gutierrez, W. J. Weis, L. Lior, A. Ellis, and S. Isaacs. 2002. Natural or raw almonds and an outbreak of a rare phage type of *Salmonella* Enteritidis infection. Can. Commun. Dis. Rep. 28:97-99.
- Chang SS, Han A, Reyes-De-Corcuera J, Powers J, Kang DH. 2010. Evaluation of steam pasteurization in controlling *Salmonella* serotype Enteritidis on raw almond surfaces. Lett Appl Microbiol 50:393-8.
- Christian, J.H.B. 1963. Water activity and the growth of microorganisms. In Recent Advances in Food Science, ed. J.M. Leitch and D.N. Rhodes, 3: 248–255. London: Butterworths.
- Craven, P. C., Mackel D. C., Baine W.B., Barker W. H., and Gangarosa E.J.. 1975. International outbreak of *Salmonella* Eastbourne infection traced to contaminated chocolate. Lancet 1:788-792.
- Cuervo MP. 2011. Comparison of current almond pasteurization methods and electron beam irradiation as an alternative. [DPhil dissertation]. College Station, TX: Texas A&M Univ. Available from: Texas A&M University Repository.
- Danyluk MD, Uesugi AR and Harris L. 2005. Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. J Food Prot 68:1613-22.
- Danyluk MD, Harris LJ, Schafner DW. 2006. Monte carlo simulations assessing the risk of salmonellosis from consumption of almonds. J Food Prot 69:1594-9.

- Danyluk M, Jones TM, Abd SJ, Schlitt-Dittrich F, Jacobs M, Harris LJ. 2007a. Prevalence and amounts of *Salmonella* found on raw California almonds. J Food Prot 70:820-7.
- Danyluk MD, Harris LJ, Sperber WH. 2007b. Nuts and cereals. In: Doyle MP and Beuchat LR, editors. Food Microbiology: Fundamentals and frontiers. Washington, D.C.: American Society for Microbiology Press. p 171-83.
- Danyluk M, Brandl MT, Harris L. 2008a. Migration of *Salmonella* Enteritidis phage type 30 through almond hulls and shells. J Food Protect 71:397-401.
- Danyluk M, Nozawa-Inoue M, Hristova K, Scow K, Lampinen B, Harris L. 2008b. Survival and growth of *Salmonella* Enteritidis PT 30 in almond orchard soils. J Appl Microbiol 104:1391-9.
- De Rezende, C. L. E., E. T. Mallinson, A. Gupta, and S. W. Joseph. 2001. *Salmonella* spp. are affected by different levels of water activity in closed microcosmos. J Ind. Microbiol Biotechnol 26:222-225.
- D'Aoust, J.-Y. 1977. *Salmonella* and the chocolate industry: A review. J. Food Prot. 40: 718-727.
- Dickson J. 2001. Radiation inactivation of microorganisms In: Molins R. Food irradiation: principles and application. Danvers MA: John Wiley & Sons, Inc. p 23-36.
- [Accessed 2013 May 18]. Available http://www.knovel.com/web/portal/browse/display?_EXT_KNOVEL_DISPLAY_bookid=1048&VerticalID=0
- Da Silva, F. V. M., & Gibbs, P. A. 2009. Principles of thermal processing: pasteurization. In Simpson (Ed.), Engineering aspects of thermal food processing (pp. 13–49). Boca Raton: Taylor & Francis.

- Deng S, Ruan R, Mok CK, Huang G, Lin X, Chen P. 2007. Inactivation of *Escherichia coli* on almonds using nonthermal plasma. J Food Sci 72:M62-M6.
- De Jong, B., Andersson, Y., Giesecke, J., Hellström, L., Stamer, U., and Wollin, R. 2001. *Salmonella* Typhimurium outbreak in Sweden from contaminated jars of helva (or halva). Euro Surveill. 5:29;pii=1715. [Accessed 2013 May 18]. Available <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1715>
- Du, W.-X., S. J. Abd, K. L. McCarthy, and L. J. Harris. 2010. Reduction of *Salmonella* on inoculated almonds exposed to hot oil. J Food Prot 73:1238-1246.
- Federal Register. 2007. Almonds grown in California; outgoing quality control requirements. Federal Register, 72: 15021–15036. [Accessed 2013 May 20]. Available from: www.gpoaccess.gov/fr/retrieve.html.
- Farkas, J. 2004. Food irradiation. In A. Mozumder, & Y. Hatano (Eds.), Charged particle and photon interactions with matter (pp. 785e812). New York, Basel: Marcel Dekker, Inc.
- G. Ladizinsky. 1999. On the origin of almond. Genetic Resources and Crop Evaluation. Kluwer Academic pub. Netherlands 46:143-147.
- Gill, O. N., Sockett, P. N., Barlett, C. R. L., Vaile, M. S. B., Rowe, B., Gilbert, R. J., Dulake, C., Murell, H. C., Salmaso, S. 1983. Outbreak of *Salmonella* Napoli infection caused by contaminated chocolate bars. The Lancet, 12: 574-577.
- GMA *Salmonella* Control Task Force. 2009. Control of *Salmonella* in low-moisture foods. Grocery Manufacturers Association, Washington, D.C. [Accessed 2013 May 20]. http://www.gmaonline.org/downloads/technical-guidance-and-tools/Salmonella_guidanceannex.pdf

- Goodridge, L. D., J. Willford, and N. Kalchayanand. 2006. Destruction of *Salmonella* Enteritidis inoculated onto raw almonds by high hydrostatic pressure. *Food Res. Int.* 39:408–412.
- Harris, L. J., A. R. Uesugi, S. J. Abd, and K. L. McCarthy. 2011. Survival of *Salmonella* Enteritidis PT 30 on inoculated almond kernels in hot water treatments. *Food Res. Int.* 45:1093–1098.
- Hirmatsu, R., M. Matsumoto, K. Sakae, and Y. Miyazaki. 2005. Ability of Shiga toxin-producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry foods, *Appl. Environ. Microb.* 71:6657-6663.
- Hockin, J. C., J.-Y. D'Aoust, D. Bowering, J. H. Jessop, B. Khanna, H. Lior, and M. E. Milling. 1989. An international outbreak of *Salmonella* nima from imported chocolate. *J Food Prot* 52:51-54.
- Isaacs S, Aramini J, Ciebin B, Farrar JA, Ahmed R, Middleton D, Chandran AU, Harris J, Howes M, Chan E, Pichette AS, Campbell K, Gupta A, Lior LY, Pearce M, Clark C, Rodgers F, Jamieson F, Brophy I, Ellis A. 2005. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. *J Food Prot* 68:191-8.
- Janning, B., P. H. in't Veld, S. Notennans, and J. Kramer. 1994. Resistance of bacterial strains to dry conditions: use of anhydrous silica gel in a desiccation model system. *J. Appl. Bacteriol.* 77:319-324.
- Jay J M, Loessner MJ, Golden DA, editors. 2005. *Modern food microbiology*. New York NY: Springer Scie. 790 p.

- Jeong S, Marks BP, Orta-Ramirez A. 2009. Thermal inactivation kinetics for *Salmonella* Enteritidis PT30 on almonds subjected to moist-air convection heating. J Food Prot 72:1602-9.
- Jenkins DJ, Kendall CW, Marchie A, Parker TL, Connelly PW, Qian W, Haight JS, Faulkner D, Vidgen E, Lapsley KG, Spiller GA. 2002. Dose response of almonds on coronary heart disease risk factors: blood lipids, oxidized low-density lipoproteins, lipoprotein (a), homocysteine, and pulmonary nitric oxide: a randomized, controlled, crossover trial. Circulation 106:1327-32.
- John M. Labavitch. 2011. Almond. Pomology Department, University of California, Davis, CA. [Accessed 2013 May 20]: <http://www.ba.ars.usda.gov/hb66/157almond.pdf>
- Juven, B. J., N. A. Cox, J. S. Bailey, J. E. Thomson, O. W. Charles, and J. V. Shutze. 1984. Survival of *Salmonella* in dry food and feed. J Food Prot 47:445-448.
- Kaspar CW, Tamplin ML. 1993. Effects of temperature and salinity on the survival of *Vibrio vulnificus* in seawater and shellfish. Appl Environ Microbiol 59:2425-9.
- Kader, A.A. 1996. In-plant storage. In: Almond Production Manual, W.C. Micke (ed) Univ. of Calif., Div. Ag. and Natural Res., Pub. No. 3364, pp. 274-277.
- Kapperud, G., Gustavsen, S., Hellesnes, I., Hansen, A. H., Lassen, J., Hirn, J. Jahkola, M., Montenegro, M. A., Helmuth., R. 1990. Outbreak of *Salmonella* Typhimurium infection traced to contaminated chocolate and caused by a strain lacking the 60-megadalton virulence plasmid. J Clinical Microbi 28, 2597-2601.
- Kester DE, Ross NW. 1996. History. In: Micke WC, editor. Almond production manual. Oakland, CA: University of California. Division of Agriculture and Natural Resources. p 1-2.

- Keady, S., G. Briggs, J. Farrar, J. C. Mohle-Boetani, J. O'Connell, S. B. Werner, D. Anderson, L. Tenglesen, S. Bidols, B. Albanese, C. Gordan, E. Debess, J. Hatch, W.E. Keene, M. Plantenga, J. Tierheimer, A.L. Hackman, C.E. Rinehardt, C.H. Sandt, A. Ingram, S. Hansen, S. Hurt, M. Poulson, R. Pallipamu, J. Wicklund, C. Braden, J. Lockett, S. Van Duyne, A. Dechet, C. Smesler. 2004. Outbreak of *Salmonella* serotype enteritidis infections associated with raw almonds - United States and Canada, 2003-2004. MMWR. 53: 484-487
- Killalea, D., L. R. Ward, D. Roberts, J. de Louvois, F. Sufi, J. M. Stuart, P. G. Wall, M. Susman, M. Schwieger, P. J. Sanderson, I. S. T. Fisher, P. S. Mead, O. N. Gill, C. L. R. Bartlett, and B. Rowe. 1996. International epidemiological and microbiological study of outbreak of *Salmonella* Agona infection from a ready to eat savoury snack-I: England and Wales and the United States. Br. Med. J. 313:1105-1107.
- Kirk, M. D., C. L. Little, M. Lem, M. Fyfe, D. Genobile, A. Tan, J. Threlfall, A. Paccagenella, D. Lightfoot, H. Lyi, L. McIntyre, L. Ward, D. J. Brown, S. Surnam, and I. S. T. Fisher. 2004. An outbreak due to peanuts in their shell caused by *Salmonella* enterica serotypes Stanley and Newport – sharing molecular information to solve international outbreaks. Epidemiol Infect. 132:571-577.
- Lee S-Y, OH S-W, Chung H-J, Reyes-de-Corcuera JI, Powers JR, Kang D-H. 2006. Reduction of *Salmonella* enterica serovar Enteritidis on the surface of raw shelled almonds by exposure to steam. J Food Prot 69:591-5.
- Ledet Muller, L., Hjertqvist, M., Payne, L., Petterson, H., Olsson, A., Plym Forshell, L., Andersson, Y. 2007. Cluster of *Salmonella* Enteritidis in Sweden, 2005 - 2006 – suspected source: almonds. Eurosurveillance, 12: 153-155.

- Little, C. 2001. International outbreak of *Salmonella* Typhimurium DT104 – update from the United Kingdom. Euro Surveill. 5:1700. [Accessed 2013 May 20]:
<http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1700>
- Mattick KL, Jorgensen F, Legan JD, Lappin-Scott HM, Humphrey TJ. 2000b. Habituation of *Salmonella* spp. at reduced water activity and its effect on heat tolerance. Appl Environ Microb 66:4921-5
- MMWR, 2004. Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds --- United States and Canada, 2003—2004. Weekly report, 53; 484-487.
- Moreno M. A., Elena Castell-Perez M., Gomes C., Da Silva Paulo F. , Moreira R.G. 2007. Quality of electron beam irradiation of blueberries (*Vaccinium corymbosum* L.) at medium dose levels (1.0-3.2 kGy). LWT. Food Science and Technology, 40: 1123-1132.
- Murano, E.A, ed., 1995; Food irradiation: A Sourcebook. Ames, IA: Iowa State University Press.
- Murano Peter S. , 2010. Exploring food science and technology. Misc. Supplies.
- Pao S, Kalantari A, Huang G. 2006. Utilizing acidic sprays for eliminating *Salmonella* enterica on raw almonds. J Food Sci 71:M14-M9.
- Niemira, B.A. 2012. Cold plasma reduction of *Salmonella* and *Escherichia coli* O157:H7 on almonds using ambient pressure gases. J Food Sci. 77:M171-175.
- O’Grady, K.A., J. Powling, A. Tan, M. Valcanis, D. Lightfoot, j. Gregory, K. lalor, R. Guy, B. Ingle, R.Andrews, S. Crerar, and R. Stafford. 2001. *Salmonella* Typhimurium DT104—Australia, Europe. Archive No. 20010822.1980. [Accessed 2013 May 20]:
<http://www.promedmail.org>. Accessed 18 November 2009.

- Pan Z. , Gokhan Bingol ,Maria T. Brandl & Tara H. McHugh .2012. Review of current technologies for reduction of *Salmonella* populations on almonds, Food Bioprocess Technol 5:2046–2057.
- Pauli G.H. and Clyde A. T..1986. Irradiation of foods- an FDA perspective. Food Revi Inter, 2:79-107. [Accessed 2013 May 20]:
<http://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/UCM054571.pdf>
- Podolak, R., E. Enache, W. Stone, D. G. Black, and P. H. Elliott. 2010. Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. J Food Prot 73:1919-1936.
- Prakash A, Lim FT, Duong C, Caporaso F and Foley D. 2010. The effects of ionizing irradiation on *Salmonella* inoculated on almonds and changes in sensory properties. Radiat Phys Chem 79:502-6.
- Reil W., John m. Labavitch, and David Holmberg. 1996. Harvesting. In: Almond Production Manual, W.C. Micke (ed) Univ. of Calif., Div. Ag. and Natural Res., Pub. No. 3364, pp. 260-263.
- Rosengarten F. 1984. The book of edible nuts. New York NY: Walker and Company. 384 p.
- Scheil, W., S. Cameron, C. Dalton, C. Murray, and D. Wilson. 1998. A South Australian *Salmonella* Mbandaka outbreak investigation using a database to select controls. Aust. N.Z. J Public Health 22:536-539.
- Shohat, T., M. S. Green, D. Merom, O. N. Gill, A. Reisfeld, A. Matas, D. Blau, N. Gal, and P. E. Slater.1996. International epidemiological and microbiological study of

- outbreak of *Salmonella* Agona infection from a ready to eat savoury snack-II: Israel. Br. Med. J. 313: 1107-1109.
- Spector M.P., W. J. Kenyon.2011. Resistance and survival strategies of *Salmonella* enteric to environmental stresses. Food Research International 45:455-48.1
- Threlfall, E. J., M. D. Hampton, L. R. Ward, and B. Rowe, 1996. Application of pulsed-field gel electrophoresis to an international outbreak of *Salmonella* agona. Emerg. Infect. Dis. 2:130-132.
- Todar Kenneth, PhD.2009. *Salmonella* and salmonellosis.University of Wisconsin-Madison Department of Bacteriology. [Accessed 2013 May 20]: [Todar's Online Textbook of Bacteriology: Salmonella and Salmonellosis](#)
- Trautmann N. and Tom R. . 2001. Moisture content. The Science and Engineering of Composting. [Accessed 2013 May 30]: http://compost.css.cornell.edu/calc/moisture_content.html
- Uesugi, A. R., and L. J. Harris. 2006a. Growth of *Salmonella* Enteritidis phage type 30 in almond hull and shell slurries and survival in drying almond hulls. J Food Prot. 69:712–718.
- Uesugi AR, MD Danyluk, LJ Harris .2006b. Survival of *Salmonella* Enteritidis phage type 30 on inoculated almonds stored at 20, 4, 23, and 35oC. J Food Prot 69:1851-1857.
- Uesugi AR, M. Danyluk, RE. Mandrell, LJ Harris. 2007. Isolation of *Salmonella* Enteritidis phage type 30 from a single almond orchard over a 5-Year Period. J Food Prot 70:1784-9.
- Unicomb, L.E., G. Simmons, T. Merritt, J. Gregory, C. Nicol, P. Jelfs, M. Kirk, A. Tan, R. Thomson, J. Adamopoulos, C.L. Little, A. Currie and C.B. Dalton. 2005. Sesame

- seed products contaminated with *Salmonella*: three outbreaks associated with tahini. Epidemiol. Infect. 133: 1065-1072.
- Villa-Rojas R., Juming t., Shaojin w., Mengxiang g., Dong-h. K.,Jae-h.m., Peter g., Maria e. Sosa-m., and Aurelio l.´Pez-m.,2012. Thermal inactivation of *Salmonella* Enteritidis PT 30 in almond kernels as influenced by water activity. J Food Prot.000: 11-509.
- Werber, D., J. Dreesman, F. Feil, U. van Treeck, G. Fell, S. Ethelberg, A. M. Hauri, P. Roggentin, R. Prager, I. S. T. Fisher, S. C. Behnke, E. Bartelt, E. Weise, A. Ellis, A. Siitonen, Y.Andersson, H. Tschäpe, M. H.Kramer, and A. Ammon. 2005. International outbreak of *Salmonella* Oranienburg due to German chocolate. BMC Infect. Dis. 5:7doi:10.1186/1471-2334-5-7. [Accessed 2013 May 18]
<http://www.biomedcentral.com/1471-2334/5/7>.
- Whitworth, J. 2012. FSANZ warning follows *Salmonella* outbreak in raw almonds. Food Quality News. [Accessed 2013 May 20]: <http://www.foodqualitynews.com/Food-Alerts/FSANZ-warning-followsSalmonella-outbreak-in-raw-almonds>.
- Willford J, Mendonca A and LD Goodridge. 2008. Water pressure effectively reduces *Salmonella* Enterica serovar Enteritidis on the surface of raw almonds. J Food Prot 71:825-9.
- Wilson, M. M., and E. F. MacKenzie. 1955. Typhoid fever and salmonellosis due to the consumption of infected desiccated coconut. J. Appl. Bacteriol. 18:510-521.
- Yang, J., Bingol, G., Pan, Z., Brandl, M. T., McHugh, T. H., & Wang,H. (2010). Infrared heating for improved safety and processing efficiency of dry-roasted almonds. J Food Eng 101: 273–280.